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XI.—THE ACTION OF DILUTE ACIDS UPON CASEIN WHEN NO SOLUBLE COMPOUNDS ARE FORMED.

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In this Journal¹ it was shown that when base-free casein is suspended for a time in dilute hydrochloric acid and then filtered, the acid is not all recovered in the filtrate. According to the results then obtained, it appeared that 1 gram of casein, when thus treated, takes from solution about 5 cc. of N/100 HCl and forms with it a definite compound, which, like the casein itself, is insoluble. The method of determining the amount of acid remaining in the filtrate by titration with standard alkali did not, under the conditions employed, yield results that were more than approximate. It was thought that more satisfactory results could be obtained by measuring the changes in electrical conductivity of acids caused by treating the acids with casein.

The special object of the work described in this article was to secure additional data bearing on the question as to whether

¹ Vol. 22, 461 (1905).

casein combines with acids to form insoluble casein salts of acids, a question which has been in dispute for more than fifty years. While the basic property of proteids has long been recognized, it is within the past fifteen years that evidence has accumulated, furnishing more specific proof that acids combine with proteids to form chemical compounds, and the more intimate knowledge gained in regard to the constitution of proteids has constituted an essential part of this evidence. This view is supported by the work of the following investigators: Sjöqvist,¹ Cohnheim,² Cohnheim and Krieger,³ Erb,⁴ Bugarszky and Liebermann,⁵ Matthews,⁶ Osborne,⁷ von Rohrer,⁸ Panormoff,⁹ Hardy,¹⁰ Mellanby,¹¹ and Robertson.¹²

It was found in general that the amount of acid combining with unit mass of a given proteid in solution was not constant but dependent upon the concentrations of acid and proteid. This was explained by the hypothesis that the proteids act as weak bases and consequently their combinations with acids hydrolyze. Spiro and Pemsel¹³ concluded from the lack of constant combining proportions that the reaction between proteids and acids is not a true salt formation but a case of physical distribution of acid between proteid and solvent water. In reaching their conclusions, however, they appear to have overlooked the effect of the hydrolysis which is to be expected from salts of weak bases, and the weight of evidence inclines entirely toward the view that proteids form chemical compounds with acids.

In studying the action of acids on proteids, various methods have been used by different workers in measuring the amount of free acid that disappears as such after treatment with proteid.

¹ Skand. Arch. Physiol., **6**, 277 (1894) and **6**, 255 (1895).

² Ztschr. Biol., **33**, 489 (1896).

³ Ztschr. Biol., **40**, 95 (1900).

⁴ *Ibid.*, **41**, 309 (1901).

⁵ Arch. Physiol. (Pflüger), **72**, 51 (1898).

⁶ Amer. Jour. Physiol., **7**, 445 (1898).

⁷ J. Am. Chem. Soc., **24**, 39 (1902).

⁸ Arch. Physiol. (Pflüger), **90**, 368 (1902).

⁹ Jour. Russ. Physiol. Chem. Gesell., **31**, 556.

¹⁰ Jour. Physiol., **33**, 333 (1905).

¹¹ *Ibid.*, **33**, 373 (1905).

¹² Jour. Biol. Chem., **2**, 317 (1907).

¹³ Arch. Physiol. Chem., **26**, 233 (1898-9).

In the case of a soluble proteid-acid product, the compound may be precipitated and the quantity of acid that is held in the precipitate or left in the solution determined by ordinary chemical methods. Titration of the solution has been the method usually employed. As precipitants, Cohnheim and also Spiro and Pemsel used ammonium sulphate. Later, Cohnheim and Krieger introduced the use of calcium phosphotungstate. Erb also used this method, and his results led to the conclusion that proteid-acid salts hydrolyze more completely as their concentrations increase, a behavior directly opposite to that of known hydrolyzible salts. Von Rohrer, however, showed that the calcium phosphotungstate method could not give accurate results, because the phosphotungstic acid, like phosphoric, renders accurate titration impossible. He showed that picric acid and dipotassium mercuric tetraiodide (K_2HgI_4) precipitate acid albumin containing an unvarying equivalent of acid independent of the excess or kind of acid, also of the temperature and of the precipitant used. He explains this on the ground that, while an equilibrium exists between the acid-proteid ($Alb.Cl$) and free proteid ($Alb.OH$) — $Alb.Cl + HOH \rightleftharpoons Alb.OH + HCl$ —only the acid-proteid is sufficiently dissociated to react with the precipitant. As this is precipitated and the equilibrium disturbed, the reaction goes from right to left, forming more $Alb.Cl$, which in turn is thrown down, until no proteid is left in solution, the precipitate being entirely unhydrolyzed acid-proteid. This method gave the acid equivalents of the proteids but, as von Rohrer points out, physico-chemical methods must be employed in order to ascertain the degree of hydrolysis of the proteid and the amount of acid it holds as it exists in solution.

Several physico-chemical methods have been employed to measure the acid combined when no precipitation occurs. Sjöqvist used the method of electrical conductivity. Bugarszky and Liebermann measured, by means of the electromotive force of concentration cells, containing acid, the changes in concentration of the latter caused by contact with proteid; they also measured the change in freezing point due to the addition of various amounts of proteid to dilute acid. Cohnheim meas-

ured the free acid by the velocity of the catalysis of cane sugar.

However, in the work done by most of the investigators mentioned above, the compounds of proteids and acids which they studied are soluble, unlike the insoluble substance formed by treating casein with dilute acids. Osborne¹ has studied the action of hydrochloric acid upon neutral edestin suspended in water, concluding that it forms a monochloride insoluble in water. Leo² states that fibrin forms a series of insoluble compounds with hydrochloric acid. Laxa³ and Richet⁴ are, so far as we know, the only workers who have worked with casein proceeding upon lines similar to our previous work. Laxa regards the insoluble substance formed by treating casein with lactic acid as a case of chemical combination. He concludes that lactates of casein which contain 1 per cent of lactic acid or less are insoluble in water, while lactates of casein with a higher content of acid are soluble.

When the proteid-acid substance is insoluble, as in the case of casein with dilute acids, the amount of acid taken up by casein may be measured by determining, through titration, how much acid remains in the filtrate after the removal of the insoluble casein-acid substance. The titration method, as used in previous work, gives misleading results, unless the conditions of treatment are such that no casein goes into solution. In case of solution, the amount of acid in the filtrate, measured as free acid, is too high, since the titration represents not only the acid that has not been taken up by insoluble casein but also that which has combined to form soluble compounds, and, in addition, the acidity of dissolved proteid itself. When the work previously published was done, the ease with which casein dissolves in very dilute acids was not fully appreciated, and the conditions then employed permitted some solution, as we have since learned by repeating some of the former work.

When no solution occurs and conditions permit the use of acids sufficiently concentrated for accurate titration, results by titration agree closely with those obtained by measuring

¹ J. Am. Chem. Soc., **24**, 39 (1902).

² Z. physiol. Chem., **46**, 286 (1905).

³ Milchw. Zentbl., **1**, 538 (1905)

⁴ Compt. rend. Soc. Biol. (Paris), **80**, 650 (1906).

electrical conductivity. In order to avoid solution, it was necessary to use dilutions as high as $N/1000$ in the case of HCl or to have the reaction occur at low temperature. In the case of high dilution, accurate titration was impossible, even when solution was prevented, and therefore conductivity measurements were made in all cases as the basis of our work.

When we began the work embodied in this article, we expected simply to measure more accurately the amount of acid that would combine with 1 gram of casein to form a definite compound insoluble in water, but we soon found the problem less simple than we had supposed. In one set of experiments, we treated varying amounts of casein with a fixed amount of acid, the larger amounts of casein being, theoretically, more than sufficient to take up all the acid judging from previous results. However, we found that some free acid remained in the filtrate in every case and, moreover, the acid was taken up in different proportions by the casein. Also, in treating casein with acids of different concentrations, it was found that the amount taken up varied with the different concentrations of solution. The phenomena were such as to suggest something else than simple definite chemical combination, and our study practically resolved itself into an effort to decide what hypothesis is best supported by facts, whether that of (1) definite chemical combination accompanied by hydrolysis, or (2) solution of acid in proteid, or (3) adsorption.

In order to obtain data which might be utilized in coming to a decision in regard to the kind of action involved, the behavior of casein was studied (1) with acids of different dissociating power, (2) with acids of different concentrations, (3) for different periods of time, (4) at different temperatures, and (5) with dilute solutions of neutral salts.

The acids used were hydrochloric, lactic, sulphuric and acetic. The concentrations of the acids used were approximately $N/125$, $N/500$, $N/1000$ and $N/2000$.

The periods of time used for keeping the casein and acid in contact varied from one minute to forty-eight hours; the periods usually employed were 5, 15 and 45 minutes, 1½, 3

and 6 hours, in the case of experiments at room temperature or higher, while additional periods of 12, 24 and sometimes 48 hours were used in the case of experiments at 0°. The temperatures used were 0°, about 25° (room temperature), and 45°. The study of hydrochloric and sulphuric acids was made to cover more details than in the case of the other acids.

DESCRIPTION OF METHODS, APPARATUS AND REAGENTS
USED.

Method of Preparing Casein.

To 1 liter of fresh, separator skim-milk, we add about 6 liters of distilled water and enough dilute acetic acid (10 to 15 cc. of strong acid diluted with water to a liter) to cause complete precipitation of casein, avoiding any marked excess of acid. The mixture is kept vigorously agitated while the acid is being gradually added. The precipitate is allowed to settle, the supernatant liquid is decanted or siphoned off, after which the precipitate is washed with copious amounts of distilled water, until the wash-water no longer shows an acid reaction to litmus. The precipitate is then treated with just enough dilute ammonia water (about 5 cc. of strong ammonia diluted to a liter) to dissolve the casein, forming a solution neutral to litmus. The solution is diluted to about 6 liters with distilled water and reprecipitated by dilute acetic acid. Much less acid is required for the second and subsequent precipitations than for the first. The precipitate is washed free from acid as before and then redissolved in dilute ammonia. Reprecipitation and redissolving should be performed five or six times. The casein should not be allowed to stand longer than necessary in contact with either acid or alkali. The final filtration and washing are completed on a Buchner funnel, the precipitate being washed until free from acid. The washed precipitate is then suspended in 1 liter of N/1000 HCl and agitated for two hours, in order to remove any remaining inorganic salts as completely as possible. Two treatments of this kind are given. The casein is finally washed until free from hydrochloric acid and is then agitated for two or three hours with a liter of very pure water (showing a conductivity not

greater than 1.5 to 1.8×10^{-6} , the operation being repeated two or three times until the filtrate shows an increase of not more than 1 or 2×10^{-6} in conductivity, as compared with the conductivity of the wash-water used. The precipitate is then treated with about a liter each of strong alcohol and ether (these reagents should show no conductivity when mixed with pure water) in order to remove any fat that may adhere to the casein. The precipitate is then dried at room temperature, ground fine in a mortar and finally dried at 45° to 50° , until the moisture content is reduced to 3 or 4 per cent. Casein thus prepared by us had an ash content of 0.25 to 0.30 per cent. We used this method of drying because, according to Laqueur and Sackur,¹ moisture can be completely removed from casein only by heating to a temperature that may alter the nature of the proteid. Correction is made for moisture, so that the amounts used in our work represent water-free casein.

It is important that the casein, when suspended in water, shall show little or no conductivity. With sufficient care, casein can be prepared that has practically no conductivity. Hardy² speaks of the impossibility of obtaining suspensions of globulin free from conductivity.

The thorough treatment of the casein with dilute hydrochloric acid and water near the end of the process of preparation rendered it probable that no salts would be left in the casein in a form capable of vitiating results by dissolving or reacting with the acids used under the conditions of experiment. Proof of this was afforded by the agreement of conductivity and titration results in experiments made under conditions such that solution of proteid was avoided, as well as by agreement of the equilibria obtained by different methods of treatment (see Fig. 3 and Table XVIII. for examples).

General Outline of Method of Measuring Acidity.

The method of finding the amount of acid taken up by a given weight of casein was, briefly stated, as follows: The casein was agitated in a Jena Erlenmeyer flask with a measured volume of acid of known strength for a given period of time,

¹ Beitr. Chem. Physiol. u. Path., **3**, 206 (1903).

² Jour. Physiol., **23**, 373 (1905).

after which it was filtered, and the amount of acid taken up by the casein was ascertained by means of the decrease in the conductivity of the solution. In a part of the work, titration results with $N/100$ NaOH were obtained for comparison with the conductivity results.

The details of the method will be considered in the following order: (1) preparation of flasks, (2) preparation of standard solutions, (3) measuring solutions for use, (4) preparation of conductivity water, (5) methods of agitation, (6) introducing casein into the acid solution, (7) filtration, (8) method of measuring conductivity, (9) data illustrating accuracy of method, and (10) calculating conductivity results in terms of concentrations of acid.

Preparation of Flasks.

Jena Erlenmeyer flasks holding 200 cc. were used in holding the acid and casein for reaction. It is important to use glassware which on contact with solutions will not affect the conductivity. Jena glassware was found to meet this requirement, and it was employed in all operations where solutions were in contact with glassware for any considerable period of time. Before being used, the flasks were carefully cleaned, rinsed with distilled water, then with conductivity water, and finally dried in a steam oven.

Rubber stoppers, thinly coated with paraffin, were used in these flasks; the stoppers were thoroughly rinsed with conductivity water and then dried on clean filter paper before being used. A test of the effect of paraffin showed it to be practically without influence upon the conductivity of water.

Preparation of Standard Solutions of Acids.

A solution of $N/10$ HCl was standardized by the official silver chloride method.¹ A solution of $N/10$ NaOH was then prepared by titration. By means of this, $N/10$ solutions of other acids were prepared. The acetic and lactic acids used were of the highest quality manufactured by Kahlbaum; the hydrochloric and sulphuric acids were of the purest grade made by

¹ Bulletin No. 46, Bureau of Chem., U. S. Dept. Agr.

Baker and Adamson. Only water of less than 2.2×10^{-8} conductivity was used in making the standard preparations of acids.

Measuring Solutions for Use.

In measuring the solutions, carefully calibrated pipettes were used and the solutions were delivered into the flasks with the smallest possible amount of exposure to air.

Preparation of Conductivity Water.

Water of conductivity as low as 1×10^{-8} was prepared by treating ordinary distilled water with a mixture of sulphuric acid and potassium bichromate and then distilling. To a 12-gallon carboy of ordinary distilled water (of conductivity equal to 15 to 18×10^{-8}) we added in water solution 20 grams of potassium bichromate and 20 cc. of concentrated sulphuric acid, and then allowed the water thus treated to stand about twenty-four hours before distilling. Distillation was performed with a retort of Jena glass, arranged in the manner described by Hulett.¹ Four liters of the treated water were placed in the retort and the first three distilling over were collected in a Jena glass flask. The conductivity of the first liter of distillate was generally 1.0 to 1.2×10^{-8} , and of the third, 1.5 to 1.8×10^{-8} . A glass condensing tube, after several liters had been distilled through it, gave as pure water as we were able to get with a block-tin tube. It was found unnecessary to redistil the water from barium hydroxide since the laboratory distilled water appeared to contain practically no carbon dioxide.

Introducing Casein into Acid Solution.

The desired amount of acid is first delivered into the reaction flask and then to this is added the weighed amount of casein. In the experiments carried on at 45° and 0° , the acid solution in the flask was allowed to come to the desired temperature before the casein was introduced.

Methods of Agitating the Mixture of Casein and Acid.

In most of the experiments made at room temperature,

¹ Z. physik. Chem., **21**, 297 (1896).

we placed the flasks in a revolving apparatus, which kept the casein in constant motion throughout the acid solution. In the experiments made at 0° and 45° , the agitation was performed by occasional shaking by hand. When the mixtures of casein and acid were shaken only at intervals, the reaction rate was somewhat slower and less regular than when agitation was continuous, the irregularity being shown by points lying noticeably off their curves occasionally, but the difference was not great and, of course, the equilibrium finally reached was not altered.

Method of Filtration.

When the casein has been in contact with the acid as long as desired, the liquid is filtered into a 200 cc. Jena flask through a platinum Gooch crucible. The receiving flasks are cleaned and dried in the manner described above. The Gooch crucibles, before use, are rinsed with conductivity water; then a thin mat of asbestos, previously suspended in conductivity water, is formed in the bottom of each. Before use the whole is dried in order to prevent any dilution of the filtrate by adherent water. To insure the filtrate further against possible contamination, the precaution is taken to rinse the receiving flask with two portions, of a few cubic centimeters each, of the filtrate first drawn through, these rinsings being thrown away. In this way, 100 cc. of liquid can be filtered in less than a minute, thus reducing to a minimum the exposure of the solution to air. Water having a conductivity of 1.5×10^{-6} increased only 0.2 to 0.3×10^{-6} after being passed through such a filter in a blank experiment.

Method of Measuring the Conductivity of the Filtrate.

The flask containing the filtrate is closed at once after filtration by a paraffined rubber stopper, prepared in the manner previously described; it is then placed in the thermostat at 25° and allowed to remain until the solution has reached this temperature, usually about an hour. The electrical conductivity is then measured in an ordinary Ostwald or Arrhenius cell.

After the filtrates in a number of flasks have been brought to the temperature of the thermostat, the conductivities can be taken in rapid succession. We found the following method of manipulation to combine satisfactorily accuracy and rapidity in running a series of measurements: The cell and electrodes are dried with clean filter paper after examining each filtrate, rinsed with the next filtrate to be tested, and then placed in the thermostat, after which some of the solution to be tested is placed in the cell. After the resistance is taken, the solution is stirred with the electrodes a short time to make sure that the solution has reached the temperature of the bath. We rarely observed any change in the second reading.

The reading is then taken with care, using in the resistance box a resistance which brought the knife contact near the center of the bridge, where the error is least.¹ The solution in the cell is replaced by a second portion from the same flask and the reading repeated. The two readings are usually identical. In case of disagreement, a third portion of solution is used, which practically always agrees with the second. In the case of the last portion used, three readings are taken at different parts on the bridge. The results of the readings usually agree within a limit of one part in a thousand.

All measurements of conductivity were taken with a calibrated Wheatstone bridge by the usual Kohlrausch method at a temperature of $25^{\circ} \pm 0.03^{\circ}$, maintained in a thermostat by an Ostwald regulator.

The capacities of the Ostwald and Arrhenius cells were determined and frequently checked with N/50 and N/500 KCl solutions, respectively. The solutions were made by successive dilution from a standard normal solution having a specific gravity at 18° of 1.04488 (water at $4^{\circ} = 1$). The potassium chloride had been recrystallized four times from solution in conductivity water and then finally fused.

Data Illustrating Accuracy of Method Used in Experiment.

Results obtained by the method employed could be closely duplicated when the conditions of experiment were uniform.

¹ Kohlrausch: *Leit. d. Elek.*, p. 43.

For example, four portions of 100 cc. each of N/125 lactic acid were treated under constant agitation for twenty minutes, each with 1 gram of casein. The percentages of acid taken up, as shown by measuring the conductivities of the filtrates, were 29.73, 29.94, 29.64, and 29.85. The agreement of these figures may be regarded as satisfactory when we consider the fact that the reaction is still proceeding quite rapidly at the end of twenty minutes, and that the exact time of contact was not quite the same in each case, owing to the interval required for removing the flasks from the shaker and filtering. As the reaction approaches equilibrium, the proportion of error decreases.

Methods of Calculating Equivalent Concentrations of Dilute Acids from Conductivities.

It is important, of course, to state the results of conductivity measurements in terms expressing equivalent amounts of acid. The method of Kohlrausch,¹ while convenient when only a few calculations are needed, was found unwieldy for our work, even with the mineral acids. In the case of the weak organic acids, the method was practically useless, owing to the number of approximations required in each calculation before reaching an accurate result. Consequently, we worked out two simpler methods, one for the mineral acids and one for the organic, which enabled us to make the necessary calculations with comparative ease and rapidity.

Method for Sulphuric and Hydrochloric Acids.—The purely graphical method is simplest in principle, but involves the laborious preparation of very accurate curves. The purely mathematical calculation is, as already stated, unwieldy. A combination, however, of simple approximate calculation with a relatively small graphical correction avoids both these difficulties. For the mineral acids we used the formula, $x = \frac{c}{R - \Delta}$; $x = \eta \times 10^6$ = the concentration of the acid based on that of N/1000 as unity; R = the resistance of the acid in the cell; Δ = a graphic correction to R. The

¹ *Leit. d. Elek.*, p. 130.

formula is based on the fact that, for electrolytes as strong as hydrochloric and sulphuric acids, dissociation is sufficiently complete at dilutions above $N/125$ to make the resistance approximately inversely proportional to the concentration.

If the proportion were exact, the equation $x = \frac{c}{R}$ would hold good. However, as the concentration increases from the point where dissociation is not complete, R becomes too large to fit the equation exactly, the proportionate excess increasing with the concentration. By subtracting this excess, Δ ,

from R , we have the equation $x = \frac{c}{R - \Delta}$, which is exact.

The value of Δ can easily be determined with accuracy sufficient for results correct to one pro mil. by graphic interpolation, plotting values of R as abscissae and those of Δ as ordinates. Of course, one can use conductivities as easily as resistances, employing the formula, $x = c(k - \Delta)$; k = conductivity; but more convenient curves are obtained by the use of resistances. After finding the values of c and Δ for a cell of capacity K , the formula can be used with a cell of capacity K' by multiplying c and Δ by $\frac{K'}{K}$.

In the case of sulphuric acid, the range of dilution ($N/125$ to $N/8000$) was so great that it was found convenient to use two cells and construct a curve for the range over which each was employed. The curves include the correction for the influence of water, since water of the same character and conductivity was employed in finding the values of Δ and in the subsequent experiments. This is of advantage, because, when working with mineral acids and bases, it is doubtful whether subtracting the conductivity of the water gives correct results. Ostwald favors ignoring the effect of the water since the impurity may be of such a nature as even to lower the conductivity.¹ Kohlrausch,² on the other hand, remarks in discussing this plan: "So steht ein solches Verfahren vollständig in der Luft."

The value of Δ for hydrochloric acid was determined from

¹ "Physico-chemical Measurements," translated by Walker, p. 235.

² *Leit. d. Elek.*, p. 92.

the table given below. The series of resistances was taken by repeated dilutions of the solution in the cell by means of calibrated pipettes, the series being checked by repetition and by duplicating the more dilute solutions with those made directly from N/105.8.

Table I.—Showing Values of Δ for Hydrochloric Acid.

Equivalent volume. <i>v</i> .	$x = \eta \times 10^8$.	R.	$\frac{0.5905}{x} \times 1698$ = R propor- tional.	R—R propor- tional = Δ .
105.8	9.448	107.7	106.1	1.6
211.7	4.724	213.1	212.2	0.9
423.4	2.362	425.2	424.5	0.7
846.8	1.181	849.0	849.0	0.0
1694.0	0.5905	1698.0	1698.0	0.0

Calculating the constant of the formula from the resistance at the highest dilution, we have $c = x \times R = 0.5905 \times 1698 = 1003$, which gives, as the formula,

$$x = \frac{1003}{R - \Delta} = \frac{2378 \text{ K}}{R - \Delta \text{ (corrected for K)}}$$

in which K equals capacity of cell based on the $\frac{1}{\text{ohm cm.}}$ unit of conductivity for electrolytes.

Similarly, the formula for sulphuric acid is found to be $x = \frac{2415 \text{ K}}{R - \Delta}$, the values of Δ being given below in Table II.

For general use with cells of varying capacities, it is convenient to prepare a table with values of R and Δ reduced to fit a cell of capacity equal to 1. The values of R and Δ are then transposed to fit a cell of any capacity, K', simply by multiplying them all by K', since $\frac{K'}{K} = K'$. The values for sulphuric acid for a cell of unit capacity are given in the following table (conductivity of water = 1.4×10^{-6}).

Table II.—Showing Values of R and Δ for Sulphuric Acid with Cell of Unit Capacity.

V.	$x = \eta \times 10^6$.	R.	Δ .
125	8.0	362.3	60.3
250	4.0	678.4	74.4
500	2.0	1290.0	82.0
1000	1.0	2498.0	82.0
2000	0.5	4888.0	57.0
4000	0.25	9662.0	00.0
8000	0.125	19320.0	00.0

The values of Δ_{25}° calculated for sulphuric and hydrochloric acids from the resistances given, after correcting for conductivity of water, agree within a fraction of a per cent with those of Kohlrausch¹ as corrected by the temperature coefficients of the two acids.²

Method for Lactic and Acetic Acids.—The method used in calculating the conductivity results obtained with lactic and acetic acids into their acid equivalents was, like that employed for the mineral acids, a combination of approximate calculation and graphic correction, the maxima of such corrections being 1.9 per cent for acetic acid and 5 per cent for lactic acid.

The purely empirical but convenient method of approximate calculation was based on the following data: Representing the resistance of N/125 acid as R_{125} , etc., it was found for lactic acid that $R_{250} = 1.50R_{125}$, while $R_{2000} = 1.58R_{1000}$, intermediate ratios ranging between 1.50 and 1.58. The percentage increase caused by doubling the dilution is fairly constant, varying from 50 to 58. For acetic acid the variation is even less. Thus, $R_{250} = 1.44R_{125}$ and $R_{2000} = 1.45R_{1000}$. Increasing by 100 per cent the dilution of any solution between N/125 and N/1000 concentrations results in an increase of from 44 to 45 per cent in the resistance. In general, the ratio between the percentage increase in dilution and the percentage increase in resistance is nearly constant. This relation is expressed by the following simple formula for use in calculation:

¹ *Leit. d. Elek.*, p. 160.

² *Ibid.*, p. 199.

$$(1) \log x = \log x_0 - c(\log R - \log R_0);$$

$$(2) \quad = \log x_0 - c(\log k_0 - \log k).$$

These two forms make it possible to calculate x from either resistance (1) or conductivity (2). The value of c is determined from either resistance or conductivities:

$$c = \frac{\log x_0 - \log x_d}{\log R_d - \log R_0} = \frac{\log x_0 - \log x_d}{\log k_0 - \log k_d}.$$

$x = \eta \cdot 10^8$ = the concentration sought (= 1 for N / 1000 concentration).

x_0 = concentration of the strongest solution to which the formula is applied (N/125 in present case).

x_d = concentration of the most dilute solution to which the formula is applied (N/2000 in present case).

R , R_0 and R_d are the corresponding resistances, while k , k_0 and k_d are the corresponding specific conductivities (most conveniently multiplied by 10^8 for use).

The formula was derived as follows: Assuming the constancy of the ratio between the percentage increments in resistance, R , and in dilution (or equivalent volume), V , respectively, we have,

$$(1) \frac{dV}{V} = c \frac{dR}{R}.$$

Integrating, we have,

$$(2) \ln V = c \ln R + \text{constant},$$

$$\text{const.} = \ln V_0 - c \ln R_0;$$

$$(3) \ln V = \ln V_0 + c (\ln R - \ln R_0).$$

Since $x = V^{-1}$, $\ln V = -\ln x$, we have from (3),

$$(4) \ln x = \ln x_0 - c(\ln R - \ln R_0);$$

$$\frac{R}{R_0} = \frac{k_0}{k} \therefore \ln R - \ln R_0 = \ln k_0 - \ln k.$$

Substituting $(\ln x_0 - \ln k)$ for $(\ln R - \ln R_0)$ in (4), we have

$$(5) \ln x = \ln x_0 - c(\ln k_0 - \ln k).$$

As all terms are logarithmic and require multiplication by

the same modulus to change to Briggs' logarithm, equations (4) and (5) hold without change for ordinary logarithms and constitute the formula already given. The determination of the constant of integration from values of V_0 and R_0 renders the equation exact for a solution of x_0 concentration, while the determination of c from values x_d and R_d render it exact for a solution of concentration x_d . This formula, with graphic correction, can be employed for mineral as well as organic electrolytes, though not so simple as the one previously given.

The following values were obtained for the two acids:

	x_0 .	x_d .	R_0 .	R_d .
Lactic acid	8.0	0.5	2036 K	11,610 K
Acetic "	8.0	0.5	6624 K	28,870 K

K = capacity of cell.

From these values the constants of the two formulas were obtained:

	$\log x_0$.	c .	$\log R_0$.
Lactic acid	0.9031	1.592	$4.0649 + \log K$
Acetic "	0.9031	1.882	$4.4605 + \log K$

The formulas are exact for the concentrations selected as limits ($N/125$ and $N/2000$) and approximate for intermediate points. The corrections having been determined at a few of these points, the remaining ones were graphically interpolated, plotting as abscissae values of x calculated by the formulas, and as ordinates, the corresponding corrections. These corrections, like those in the formula used for mineral acids, include those due to the influence of water. The conductivity of the latter, for all work with lactic and acetic acids, varied from 1.2×10^{-6} to 1.7×10^{-6} .

Table III.—Showing Corrections to Formulas for Lactic and Acetic Acids.

Equivalent volume.	$v, \quad x = \eta.10^6.$	x calculated from resistance by formula.		Corrections to x calculated.	
		Lactic.	Acetic.	Lactic.	Acetic.
	125	8.000	8.000	± 0.000	± 0.000
	250	4.140	4.025	-0.140	-0.025
	500	2.102	2.038	-0.102	-0.038
	1000	1.042	1.016	-0.042	-0.016
	2000	0.500	0.500	± 0.000	± 0.000

The series of resistances, from which the formula constants and intermediate corrections were calculated in the case of these two acids, were taken in the usual manner by dilution in the cell, checking the results by duplicates of the higher dilutions made directly from the lowest, and by running duplicate series.

The values found for the molecular conductivity of lactic acid, after making correction for the conductivity of the water, are considerably higher than those given by Ostwald.¹ We are unable to account for this difference, since we observed all requisite precautions, using Kahlbaum's C. P. acid and preparing fresh N/10 solutions with which to run duplicate series.

In taking the resistance of lactic acid, it was found necessary to heat the electrodes, as recommended by Whetham,² changing the platinum black to gray, before we could obtain accurate readings.

EXPERIMENTAL RESULTS.

While the main purpose of our work was to study the action of dilute acids upon casein when no soluble compounds are formed, it became necessary to ascertain the conditions under which soluble compounds are formed, in order to avoid such solution. We shall, therefore, consider this phase of our work first.

The Solubility of Casein in Dilute Acids.

The ready solubility of casein in acids of N/10 or N/20 concentration, especially hydrochloric acid, has been a familiar fact for a long time, but little has been done in studying the solubility of casein in acids much above N/100 dilution. Our work shows that casein is appreciably dissolved at 25° by hydrochloric and lactic acids of N/1000 dilution and by N/125 acetic acid. In all cases the solubility is markedly increased by increase of temperature.

Evidence of solution can be furnished by the following observations:

¹ *Leit. d. Elek.*, p. 179, Kohlrausch.

² *Phil. Trans.*, 194, 330 (1900).

(1) *Viscosity of Solution.*—After being treated with casein by the method described on p. 388, acids show marked viscosity. In the case of slight solution of casein, this is shown by the readiness with which foam is produced when the flask containing the filtrate is shaken, and also by the length of time the foam persists. The behavior of the foam is very different in amount and duration from that formed by agitating a solution of acid that contains no dissolved casein. In the case of more marked solution, viscosity is more readily noticed, and the liquid foams noticeably while being drawn through the filter; it also filters more slowly. In cases of very marked solution, filtration, even by suction through a thin asbestos mat, was difficult and the filtrate consisted largely of foam.

(2) *Behavior of Solution on Adding Alkali.*—The clear filtrate on titration with N/100 NaOH becomes opalescent and varies from that to milky turbidity and even to the formation of a heavy, curd-like precipitate, according to the amount of casein in solution. These phenomena appear before neutrality is reached and disappear before sufficient alkali is added to give a permanent color with phenolphthalein. In the case of precipitation, the readiness with which the precipitate dissolves varies with the rate at which the alkali is added. When the alkali is added slowly, the precipitate forms large flakes and these, once formed, redissolve quite slowly. On the other hand, if alkali is rapidly added in amount sufficient to redissolve any precipitate, the particles of casein, not having time to form large aggregations, redissolve almost instantly, so that only a transient, opaque milkiness is observed before redissolving takes place.

(3) *The Xanthoproteic Reaction.*—The presence of dissolved casein in the filtrate is readily shown by the xanthoproteic reaction. For the purpose of making our results comparable, this test was applied uniformly in the following manner: We boiled 10 cc. of the filtrate to be examined with 10 cc. of nitric acid (sp. gr. 1.42) and then added about 12.5 cc. (about 2 cc. in excess of neutrality) of a concentrated solution of sodium hydroxide. A blank test was used for comparison in cases where the result of the reaction appeared in doubt.

(4) *Deviation between Results by Titration and Conductivity Methods.*—When any casein dissolves, titration of the filtrate shows more acid than does the measurement of conductivity, because titration of the filtrate with alkali measures not only the acid not taken up by casein but also that combined with dissolved proteid, and, in addition, the acidity of the dissolved proteid itself, which in some cases may neutralize even more alkali than the free acid. On the other hand, the error caused in conductivity results by partial solution of proteid can, at most, amount to only a relatively small percentage, because the fixation of free hydrochloric acid for instance, to form a soluble proteid compound, in which the light and rapidly moving H^+ ion of hydrochloric acid is replaced by the enormously complex and sluggish proteid radical, results in the loss of most of the conducting power of the acid. Thus, the addition of sufficient egg albumin to combine with all the acid in N/20 HCl solution, forming soluble albumin hydrochloride, results in a decrease of 83 per cent in the conductivity of the solution.¹ Similarly, the combination of hydrochloric acid with globulin results in a decrease of 76 per cent in conductivity.² When solution of casein in acid occurs, the soluble acid proteid has still, undoubtedly, a low conductivity of its own, as in the two cases just mentioned. This will render somewhat too high the concentration of free acid calculated on the assumption that the combined acid forms only a compound which is, like the insoluble one, entirely non-conducting. The error will, however, be one of a small percentage, particularly when only a fraction of the casein dissolves and the greater part of the acid taken up goes to form the insoluble substance.

The titration results, on the other hand, as will be shown presently, may indicate only a small proportion of acid taken up, or even less than none. We made determinations of acid in the filtrates obtained from mixtures of casein and acid in numerous experiments in which we used concentrations varying from N/125 to N/500 HCl, employing both the titration and conductivity methods as checks upon each other.

¹ Sjögqvist: Skand. Archiv. f. Physiol., 5, 334 (1895).

² Hardy: Jour. Physiol., 23, 273 (1905).

We found that whenever the filtrate gave a pronounced proteid reaction, the titration results fell below the conductivity results and that, the more pronounced the proteid reaction, the greater was the difference between the two sets of results.

In some cases, when the decrease in conductivity showed that a large part of the hydrochloric acid had been removed from solution or combined with casein in a soluble form, titration with alkali showed that the filtrate had actually a *greater* acidity than could be accounted for by the total amount of acid originally used in the experiment. The titration caused a voluminous precipitate, and the filtrate showed an intense xanthoproteic reaction in such cases. The increased acidity was due to the acidity of the dissolved proteid itself.

There are, then, two reasons why the determinations of acidity by titration and by conductivity do not give concordant results when the solution contains dissolved proteid, the titration results being in error. First, the dissolved casein-acid compound decomposes at once in the presence of alkali, freeing the acid previously combined. Second, the dissolved proteid also combines with and neutralizes part of the alkali. The acidity of the filtrate, instead of representing only the amount of acid not taken up by the casein, equals the acid not so taken up, *plus* the acid that has combined with casein to form a soluble casein-acid compound, *plus* the acidity of the dissolved proteid. Experiment shows that the solution of a gram of casein in a given volume of acid increases by about $8\frac{1}{2}$ cc. the volume of N/10 NaOH required to neutralize the acid. This also is the amount of alkali required to dissolve and neutralize to phenolphthalein 1 gram of free casein. The acidity of the casein itself appears to be unchanged by solution in dilute acid, whether or not the nature of the casein complex is changed.

Effect of Temperature upon Solution of Proteid, and of Solution upon Titration Results.—The set of experiments described below shows clearly the effect of temperature upon the rate of solution and also the effect of solution upon the accuracy of the titration method as used by Van Slyke and Hart, Laxa and Leo. The experiments were performed in the manner already described. Flasks containing casein and hydro-

chloric acid were kept at 0° , 25° , and 45° in constant temperature baths which were shaken at intervals. The titrations were made in duplicate, agreement being within 0.1 cc. of N/100 alkali in all cases. The expression "acid fixed by casein," used in the following table, indicates acid taken from the state of solution as free hydrochloric acid, whether the union with casein be physical or chemical or both in part, as is probably the case when partial solution of casein occurs. We used 100 cc. of N/125 HCl for 1 gram of casein. The concentration of the acid used was 7.905 instead of 8, because the factor of the stock solution was a little less than unity. The weights of casein represent water-free casein, correction being made for the amount of moisture present, which was generally 3 to 4 per cent, this plan being adopted in all our work.

In the table (p. 405), the results are arranged to show:

- (1) Effect of temperature on the rate of solution of casein in acid (columns 10 and 11).
- (2) Effect of solution on accuracy of titration results (compare columns 6 and 9 with reference to 10 and 11).
- (3) Effect of solution on amount of hydrochloric acid fixed by casein. (Compare 5 hour figures for 0° , 25° and 45° , column 6, with reference to 10 and 11).

Explanation of Fig. 1.—The results given in Table IV. are expressed in the form of curves in Fig. 1. in which the curves marked "conduc" and having points marked "x" express results obtained by the conductivity method, while the curves marked "titr." and having points marked "o" express results obtained by the titration method.

The numbers associated with the points x and o express approximate degrees of solution of casein by acid, as indicated below (see table, p. 406).

The figures in the last column were obtained as follows: We dissolved 0.5 gram of casein in nitric acid and diluted to 500 cc., each cubic centimeter containing 1 mg. of proteid. Different amounts of this solution were diluted to 10 cc. each and tested by the xanthoproteic reaction. The same system of numbers (first column) will be uniformly employed hereafter to express approximate degrees of solution.

Table IV.—Action of $N/125$ HCl upon Casein (100 cc. Acid for One Gram Casein).

1.	2.	3.	Conductivity results.			Titration results.			Tests for casein in solution.	
			4.	5.	6.	7.	8.	9.	10.	11.
Temperature, degrees C.	Time of contact of casein and acid.	Resistance of solution in ohms.	$\eta \times 10^8 =$ concentration of acid ($N/1000 = 1$).	Decrease in $\eta \times 10^8$ caused by contact with casein.	$10^8 \times$ gm. equiv. = (cc. $N/1000$ HCl) fixed by one gram of casein.	Cc. $N/100$ NaOH to neutralize 25 cc. filtrate.	Decrease in η caused by contact with casein.	$10^8 \times$ gm. equiv. (= cc. $N/1000$ HCl) fixed by one gram of casein.	Appearance of filtrate on adding NaOH.	Xanthoproteic reaction color.
0°	0 min.	127.4	7.905			19.9				
0°	5 min.	186.3	5.383	2.522	252.2	13.5	6.4	256		
"	15 "	188.1	5.331	2.574	257.4	13.5	6.4	256	Clear	O
"	45 "	235.3	4.253	3.652	365.2	10.70	9.2	368	"	O
"	1 1/2 hrs.	247.0	4.050	3.855	385.5	10.35	9.55	382	Opalescent	O
"	5 "	267.0	3.744	4.161	416.1	9.55	10.35	414	Milky	"
25°	5 min.	245.3	4.079	3.826	382.6	10.60	9.30	372	Opalescent	"
"	15 "	257.4	3.901	4.004	400.4	10.45	9.45	378	Milky	"
"	45 "	273.0	3.661	4.244	424.4	10.95	8.95	348	Opaque	Medium "
"	2 1/2 hrs.	284.8	3.509	4.396	439.6	12.75	7.15	286	Precipitate	Dark "
"	5 "	302.3	3.305	4.600	460.0	16.35	3.55	142	"	Orange "
45°	5 min.	280.9	3.540	4.335	433.5	14.8	5.1	204	Opaque	Dark orange
"	15 min.	313.0	3.178	4.727	472.7	22.1	0.3	— 12	Heavy precipitate	Orange
"	45 "	323.0	3.080	4.825	482.5	23.9	4.0	— 160	"	"
"	5 hrs.	367.8	2.704	5.201	520.1	38.6	— 18.7	— 758	"	"

In Fig. I, it will be seen that at 0° there is comparatively slight solution of casein and the results obtained by the two methods, conductivity and titration coincide. At 25°, solution becomes marked, and the abnormally low curve after the first five minutes furnished by titration results is strikingly

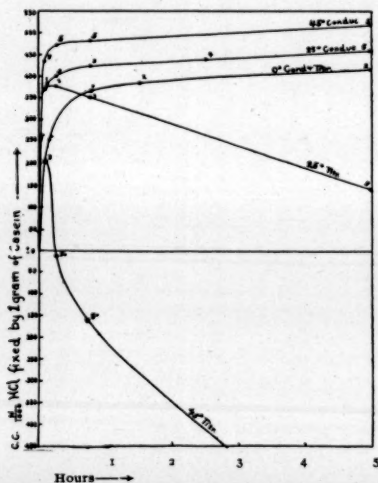


Fig. I.—Action of N/125 HCl. Showing abnormally low titration results at 25° and 45°, caused by solution of casein.

No. denoting degree of solution.	Appearance of filtrate on addition of alkali.	Color produced by xantho-proteic reaction.	Mgs. casein in 100 cc. of test solution corresponding to color.
0	clear	none	0 to 0.5
1	slightly opalescent	slightly yellow	0.6 " 0.9
2	moderately "	mod'rly "	1.0 " 3.0
3	turbid, milky	deep "	4.0 " 6.0
4	opaque, some precip.	orange	7.0 " 10.0
5	heavy precipitate	dark orange	10.0 or more
5+	" "	" "	larger amounts

apparent. At 45° the effect of solution is still more evident. In fifteen minutes the amount of dissolved casein makes the acidity of the filtrate actually greater than that of the original N/125 acid used.

The results embodied in Table IV. and in Fig. I are summarized as follows:

(1) Casein takes up hydrochloric acid from solution. At 0°, 1 gram of casein takes up from 100 cc. of N/125 HCl, without appreciable solution, the acid in about 50 cc., which is equivalent to 4 cc. N/10 acid, an amount about eight times that found by Van Slyke and Hart.¹

(2) The formation of a soluble substance from casein by the action of the acid is effected when there is an increase in temperature or in the time of contact between the acid and casein. For convenience we may term this soluble substance soluble acid-casein, without considering the question as to whether it is a salt of casein or a proteolytic product.

(3) More casein goes into solution in five minutes at 45° than in five hours at 0°.

(4) The soluble substance appears to be a combination of casein and acid. This is indicated by the fact that there is a decrease in the conductivity of the acid simultaneous with the formation of the soluble acid-casein. The decrease is so marked that there is no doubt that the formation of the soluble compound is accompanied by fixation of hydrochloric acid. Doubtless the soluble acid-casein has appreciable conductivity of its own, as already mentioned in the case of the proteids, so that even the conductivity results for the amount of acid fixed are somewhat too low.

(5) The method of determining by titration with alkali the amount of acid remaining unaffected by treatment with casein is thoroughly unreliable in the presence of soluble acid-casein.

In connection with the data here presented, it would seem that the results of the experiments above described ought to emphasize the fact that caution should be used in the determination of free acid by titration in liquids, such, for example, as gastric juice, which have been in contact with proteids.

¹ THIS JOURNAL, 33, 478 (1905).

Simon,¹ in a discussion of the subject, gives titration with sodium hydroxide, using phenolphthalein for indicator, as the method for estimating the acidity of the gastric contents, and he also states that the acidity is increased by the ingestion of albuminous food. This would naturally be found so by the titration method, because the proteid in solution would neutralize part of the alkali used in titration and thus increase the amount required.

Effect of Temperature and Concentration upon the Rate at which Acids Dissolve Casein.—We present below in Tables V., VI., and VII. results indicating the effect of temperature and concentration of acid upon the rate at which acids convert casein into solution. The acids used in our study are hydrochloric, lactic, sulphuric and acetic; the temperatures, 0°, 25° and 45°; and the concentrations, N/125, N/500, N/1000 and N/2000. The numbers in the tables indicate degrees of solution, as explained on p. 404 in connection with Fig. I. The figures on the left in each column express degrees of solution as shown by the appearance of the filtrate when treated with sodium hydroxide; those at the right indicate the results of the xanthoproteic test.

The results embodied in Tables V., VI., and VII. are summarized as follows:

(1) Comparing hydrochloric and lactic acids, it is seen that these two acids have about the same solvent power at 0° with the higher dilutions, but with N/125 acid the solvent power of the lactic acid is somewhat greater. Increase of temperature increases the solvent effect of lactic acid less than that of hydrochloric.

(2) Sulphuric acid dissolves casein more slowly than does hydrochloric acid of the same strength.

(3) Acetic acid is distinguished for its comparatively slight solvent action on casein at ordinary temperatures. This fact is of interest in connection with the method of separating casein from milk by precipitation with acetic acid, the method in general use. Its use involves a more complete separation under uniform conditions, and with less solvent action, than can be attained by use of the other acids studied.

¹ "Physiological Chemistry," p. 128 (1904).

Table V.—Showing Effect of Temperature and Concentration upon Rate of Solution of Casein by Hydrochloric Acid.

Time of contact.	N/25.			N/500.			N/1000.			N/2000.		
	0°	25°	45°	0°	25°	45°	0°	25°	45°	0°	25°	45°
5 min.	0	0	1	1	3	4	0	0	0	0	0	0
15 "	0	0	3	2	5+	5+	0	0	0	0	0	1
45 "	0	0	3	3	5+	5+	0	0	0	0	0	2
1½ hrs.	2	2					0	0	1	0	0	2
2½-3 hrs.	2	2	4	4	5+	5+	1	0	1	1	3	3
5-6 "	3	2	5	5	5+	5+	1	1	3	3	5	5
12 "	3					*	2	2	4	4	5	5
24 "	4					*	3	3	5	5	5	5

* Solution nearly complete.

Table VI.—Showing Effect of Temperature and Concentration on Rate of Solution of Casein by Lactic Acid.

Time of contact.	N/125.			N/500.			N/1000.		
	0°	25°	45°	0°	25°	45°	0°	25°	45°
5 min.	0	1	1	3	2	0	0	0	0
15 "	1	1	1	1	1	1	0	0	0
45 "	2	2	3	4	4	0	0	0	1
1½ hrs.	3	2	4	4	4	0	0	0	1
3 "	3	3	4	5	0	1	0	0	1
6 "	4	3	5	5+	1	1	0	1	2
12 "	4	4	5	5+	1	1	1	1	1
24 "	4	4	2						

Table VII.—Showing Effect of Temperature and Concentration upon Rate of Solution of Casein by Sulphuric and Acetic Acids. (Figures Show Results of Xanthoproteic Test Only.)

Time of contact.	Sulphuric acid.			Acetic acid.		
	N/125.	N/500.	N/1000.	N/125.	N/500.	N/1000.
15 min.	25°	0°	25°	0°	25°	25°
45 "	0	0	0	0	0	0
3 hrs.	2	1	0	1	1	0
6 "	2	2	1	1	1	0
12 "	4	0	1	1	0	0
24 "		1	1	2	0	0

(4) In order to study the effect of the acids under consideration upon casein without dissolving appreciable amounts of casein, it is necessary to use acid solutions as dilute as N/500 to N/2000 and at temperatures not above 25° C.

Action of Neutral Salts upon the Solubility of Casein.—In order to test the effect of replacing the H^+ ion, casein was treated at room temperature with N/125 $MgSO_4$ and with N/50 KCl, the same method of treatment being used as with the acids. Even after constant agitation of casein with magnesium sulphate for six hours and with potassium chloride for twenty-eight hours, we were unable to obtain any evidence of solution of casein.

The solvent power of the dilute solution, at least of hydrochloric and sulphuric acids, appears, therefore, to depend upon the presence of H^+ ions. However, since the solvent power of lactic acid is comparable with that of the mineral acids, it suggests that, other conditions being uniform, the concentration of the H^+ ions is not the only factor. As bearing on this point, a quantitative test of the solubilities of casein in acids of variable dissociating powers is desirable.

The following figures represent the percentage of dissociation and, according to the dissociation theory, the relative concentration of the H^+ ions in solutions of the four acids of the concentrations used:

Equivalent volume.	Per cent of dissociation.			
	Hydrochloric acid.	Sulphuric acid.	Lactic acid.	Acetic acid.
125	100	82	12.2	4.6
500	100	95	22.7	9.1
1000	100	98	30.7	12.6

The figures for hydrochloric and sulphuric acids are according to Jones and Douglas;¹ those for lactic and acetic acids, from Ostwald.²

The results presented in Tables V., VI. and VII. show that hydrochloric acid has stronger solvent power than sulphuric acid and that lactic acid is stronger than acetic, results which

¹ Am. Chem. Jour., 26, 434 (1901).

² Zeit. f. Physik. Chem., 3, 174 and 191 (1889).

are in general harmony with the dissociation figures preceding as far as the two mineral and the two organic acids, taken separately, compare with each other; but the dissolving power of the two organic acids, compared with that of the mineral acids, is entirely out of proportion to the relative dissociating powers of the former. Apparently, the anions of the organic acids as well as the H^+ ions influence the solvent power. This solvent effect of the organic ions is also shown by Robertson's result.¹ While he found that N/10 solutions of the chloride, sulphate and nitrate of sodium did not dissolve casein appreciably, the acetate, propionate, butyrate and valerate all showed marked solvent action.

The solution of casein in dilute acids may be the result of the formation of a soluble casein-acid salt, similar to an amido salt, or it may be the result of the decomposition of the casein molecule, part of which is split off and gives the reaction for proteid in solution. In the latter case, we should expect some change in the properties of the undissolved portion, and this is found to be true. An attempt was made to determine the percentage of casein dissolved at 25° by N/125 HCl in three hours; the undissolved casein was collected in a Gooch crucible, washed, and dried at 100° . At the time of filtration the undissolved residue presented a swollen, gelatinous appearance and, when placed in the steam oven to dry, it completely melted, running through the perforated bottom of the crucible. The dried mass looked like dried albumin. The experiment was repeated with the same result. Casein itself can be heated to 120° or higher without visible change, and casein that has taken up acid without any solution of the proteid does not appear either to swell or gelatinize, and it can be dried in a steam oven without difficulty. When a part of the casein goes into solution, the undissolved residue appears to undergo some profound change in its properties, and this fact suggests the possibility of molecular decomposition. The scope of our work did not permit us for the time being to carry farther our study into the details of this interesting change.

¹ Jour. Biol. Chem., **2**, 338 (1907).

The Action of Hydrochloric Acid upon Casein without Solution.

In the foregoing pages we have studied the conditions under which casein goes into partial solution when treated with dilute acids, and also those under which such solution may be avoided. Working under the latter conditions, as completely as possible, we now purpose to ascertain to what extent and under what conditions casein takes up acid from dilute solutions when no casein dissolves. It has already been shown by both titration and conductivity methods (Fig. I., p. 406, curve for 0°), that when a gram of casein is shaken with 100 cc. of N/125 HCl at 0° , about one-half of the acid is removed from solution before appreciable solution of casein occurs. The fact that the acid is thus taken up to form an insoluble substance with casein being shown, the question remains: Is the taking up of acid by casein a chemical reaction and is the resulting insoluble casein-acid substance a definite chemical compound, or is the process one of adsorption? The results which follow appear to indicate that the behavior of the reaction places it in the class of adsorption reactions, of which the adsorption of dyes and dilute acids by silk forms a parallel example.¹

Our results point to the following conclusions: First, contrary to the former belief of Van Slyke and Hart, there is no definite insoluble chemical compound formed, but the amount of acid taken up by casein varies, temperature remaining constant, in proportion to the concentration of the acid in the surrounding solution. The ratio $\frac{\text{HCl in 1 gram casein}}{\text{HCl in 1 cc. H}_2\text{O}}$ is a constant under uniform conditions between the limits of concentration studied, and can be established either by acid-casein, giving up acid to the surrounding water, or vice versa, the acid going either way according to the initial concentration.

Second, the variation in the amount of acid taken up by casein does not appear to be due to the hydrolysis of a casein-acid salt but rather appears to occur in accordance with the behavior of adsorption reactions, as will be pointed out in later discussion.

¹ Schmidt: Z. physik. Chem., 18, 56 (1894). Walker and Appleyard: J. Chem. Soc., 69, 1334 (1896).

In carrying on our study, it was important to employ conditions under which the reaction reaches equilibrium without solvent action on the casein. We found it necessary to use acids not stronger than $N/125$ and to carry on the experiments at 0° in order to avoid solution before the casein had taken up all the acid it could. Even under these conditions, long standing in contact with acids usually resulted in perceptible solution of casein, but to a degree so slight that it did not appear to affect the results, except possibly in the case of the 24-hour periods with $N/125$ acid.

In the tables following, the concentration of acid in the solutions is given in terms expressing the number of cubic centimeters of $N/1000$ acid equivalent to 1 cc. of solution used. The concentration of acid in the casein is given in terms expressing the number of cubic centimeters of $N/1000$ acid taken up by 1 gram of casein.

In the third column, the figure representing the amount of $N/1000$ acid in 1 cc. of solution given for zero time, indicates the exact concentration of the acid used in the series, as determined by measurement of its conductivity. This value generally differs slightly from the figures corresponding to the normality given in the first column, because the stock solutions usually possessed factors varying somewhat from unity.

In the fourth column (concentration of acid in casein), the amount of acid taken up by 1 gram of casein in each case is calculated from the difference between the original concentration of the aqueous solution of acid and its concentration after contact with casein for the time stated, as in Table IV. The calculation is based upon the formula

$$C = \frac{D \times V}{g},$$
 in which C represents the concentration of acid in casein; D , the decrease in concentration of acid in aqueous solution caused by contact with casein; V , the volume of the solution; and g , grams of casein used.

In the sixth column, the figures express the ratio of the amount of acid in 1 gram of casein to the amount of acid in 1 cc. of the surrounding solution at the end of the period during which the acid and casein were in contact.

Table VIII.—Showing Amounts of Hydrochloric Acid Taken up at 0° by Casein in Contact with Solutions of Varying Concentration.

Proportions and approximate normality of acid used.	Duration of contact.	Concentration of acid in water (= cc. N/1000 acid in 1 cc. of solution).	Concentration of acid in casein (= cc. N/1000 acid in 1 gram casein).	Per cent of acid in original solution taken up by casein.	Ratio of acid in 1 gram casein to acid in 1 cc. water.	Degree of solution of casein.
N/2400 200 cc. for 1 gram of casein.	0	0.4168	0	0	0	
	3 hrs.	0.2848	26.4	31.7	92.7	0
	6 hrs.	0.2683	29.7	35.6	110.6	0
	12 hrs.	0.2400	35.4	42.4	147.7	0
	24 hrs.	0.2406	35.2	42.3	146.4	0
N/1000 200 cc. for 1 gram of casein.	0	0.9986	0	0	0	
	15 min.	0.8221	35.3	17.7	42.9	0
	45 "	0.8172	36.3	18.2	44.4	0
	1½ hrs.	0.7565	48.4	24.2	64.4	0
	3 hrs.	0.6631	67.1	33.6	101.3	0
	6 "	0.6313	73.4	36.7	116.2	0
	10 "	0.6112	77.5	38.8	131.3	1
	24 "	0.5915	81.4	40.7	142.8	1
N/500 200 cc. for 1 gram of casein.	0	1.986	0	0	0	
	15 min.	1.670	63.2	15.9	37.8	0
	45 "	1.570	83.2	21.0	53.0	0
	1½ hrs.	1.453	106.6	26.8	73.3	0
	3 "	1.383	120.6	32.0	93.9	1
	6 "	1.182	160.8	40.6	136.2	1
	10 "	1.176	162.4	40.9	138.0	2
	24 "	1.142	168.8	42.5	147.5	3
N/125 100 cc. for 1 gram of casein.	0	7.905	0	0	0	
	15 min.	5.331	257.4	32.6	48.2	0
	45 "	4.253	365.2	46.2	85.8	0
	1½ hrs.	4.050	385.5	48.8	95.1	2
	5 "	3.744	416.1	52.6	111.2	2
	0	7.993 ¹	0	0	0	
	12 "	3.306	468.7	58.6	141.8	3
	24 "	{ 3.188 3.195	{ 480.5 479.8	{ 60.1 60.0	{ 150.8 150.2	{ 4 4

¹ Acid of 7.993 concentration was used for last two experiments of N/125 series. The percentage of acid taken up from the N/125 solution is greater because only 100 cc. of solution were used. The ratio reached (column 6) is about the same.

The following statements summarize the results presented in Table VIII.:

(1) At 0° casein takes up increasing amounts of acid for about twenty-four hours, when equilibrium appears to be practically established, as is shown by the fact that the curves in Fig. II. approach a horizontal position when the abscissae denote that length of contact between acid and casein.

(2) The final amount of acid taken up by 1 gram of casein varies in proportion to the concentration of the surrounding acid solution, as is shown by the twenty-four hour results in the fourth column. With the range of concentrations of hydrochloric acid employed, the final amount of acid taken up by 1 gram of casein varies from the equivalent of 35.36 to 480.3 cc. N/1000 HCl, which amounts were taken up by casein in contact with solutions of acid whose final concentrations were 0.2400 and 3.192 respectively.

(3) If we divide the amount of acid contained in 1 gram of casein by the amount of acid contained in 1 cc. of the surrounding solution at equilibrium, we obtain a ratio, about 147, which is approximately constant, as shown in the sixth column. The lower value, 142.8, obtained with N/1000 acid is probably due to incomplete attainment of equilibrium. The slightly higher result in the case of N/125 acid is to be expected, since appreciable solution of proteid occurs.

(4) The acid, however small the amount used, was in no case completely taken up by casein, but the ratio, amount of acid in 1 gram casein
amount of acid in 1 cc. water, approaches an equilibrium constant, about 147, with the concentrations tested.

The results given in the fourth column of Table VIII. are expressed in Fig. II. in the form of curves, which illustrate the fact that the process by which casein takes up acid does not follow the law of definite combining proportions, and that the amount of acid fixed by a gram of casein in the presence of an excess of acid is not constant but increases with increasing concentration of acid.

Amount of Acid Taken Up by Casein Dependent upon the Concentration of Acid Present.—In order to illustrate more

fully the fact that the amount of acid taken up by 1 gram of casein is dependent, not upon the absolute amount of acid present, but upon the concentration, we give the data embodied in Table IX. The three series of experiments were carried on at different times and for different purposes, which accounts for the lack of uniformity in the time intervals used. It will

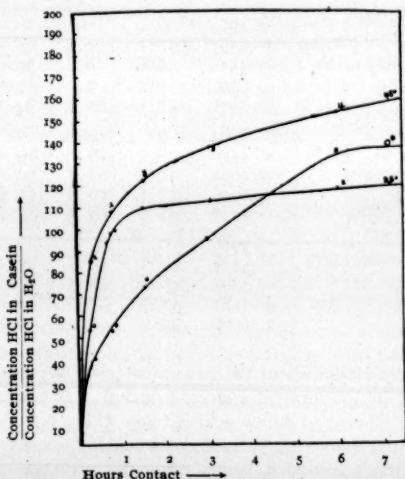


Fig. II.—Showing (1) amounts of HCl taken up at 0° by casein in contact with different concentrations; (2) rate of reaction; (3) the dependence of the final amount of acid taken up upon the concentration used; and (4) absence of definite combining proportions.

be noticed that, while the initial concentration of the acid in the third series is twice that of the others, the final concentrations are fairly comparable in all three cases, which is due to the larger relative proportion of casein used in the third series. The mixtures were shaken constantly for the periods indicated and kept at 25° .

Table IX.—Showing Relation of Amounts of Hydrochloric Acid Fixed by Casein to Concentration of Acid, at 25°.

Proportions and approximate normality of acid used.	Duration of contact.	Concentration of acid in water (= cc. N/1000 acid in 1 cc. solution).	Concentration of acid in casein (= cc. N/1000 acid in 1 gram casein).	Per cent of acid in original solution taken up by casein.	Ratio of acid in 1 gram casein to acid in 1 cc. water.	Degree of solution of casein.
Series 1	0	0.4985	0	0	0	
N/2000	30 min.	0.4597	32.1	7.8	69.9	0
828 cc. for	1 hr.	0.4550	36.0	8.7	79.1	0
1 gram of	2 hrs.	0.4507	39.7	9.6	87.8	0
casein.	4 "	0.4476	42.1	10.2	94.1	0
Series 2	0	0.4985	0	0	0	
N/2000	1 "	0.4156	33.2	16.6	79.8	0
400 cc. for	2½ "	0.4064	36.8	18.5	90.6	0
1 gram of	5 "	0.4050	37.4	18.8	92.4	0
casein.	7 "	0.4011	39.0	19.5	97.1	0
Series 3	0	0.9970	0	0	0	
N/1000	15 min.	0.5556	44.1	44.3	79.4	0
100 cc. for	50 "	0.5333	46.4	46.5	87.0	0
1 gram of	1½ hrs.	0.5181	47.9	48.0	92.4	0
casein.	3 "	0.5066	49.0	49.2	96.8	0

The results embodied in the preceding table are also shown in Fig. III. in the form of curves A and B. It is noticeable that the initial rate at which acid is taken from N/1000 solution is greater than from N/2000, as would be expected, but the two curves approach nearly the same asymptote. The points (x and o) for the two N/2000 solutions lie on the same curve A.

Extraction of Hydrochloric Acid Taken Up by Casein.—The reaction by which casein fixes acid is reversible. By treating with pure water casein that has taken up acid, part of the acid passes from the casein back into the surrounding water.

The method of performing the experiment was as follows: In weighed Jena flasks were placed 1-gram portions of casein and 100 cc. portions of N/500 HCl. The mixtures were shaken continuously for thirty minutes, in order to allow the casein to take up acid under conditions not permitting appreciable

amounts of casein to go into solution. The mixtures were then allowed to settle for about five minutes and the supernatant liquid decanted as completely as possible through weighed Gooch crucibles, into which the decantation usually carried 10 to 20 mgs. of casein. The filtrate (1) was kept for examination. The crucibles were dried and weighed in order to find out how much casein had escaped from the flask during decantation. The flasks with contents were weighed after decantation in order to find how much free acid solution adhered to the casein particles and the walls of the flask; the amount varied from 2 to 4 cc. To the casein left in each flask there was then added 100 cc. of pure water, and the mixtures were shaken for different periods of time, varying from one minute to five hours as indicated in Table XI., column J. They were then filtered again (Filtrate 2). The amount of acid in each filtrate was determined by conductivity measurements. From the first filtrate were obtained the data necessary to determine how much acid had been taken up by the casein to form the insoluble acid-casein, when shaken thirty minutes with acid. From the second filtrate were obtained the data required to determine the amount of acid extracted from the insoluble acid-casein by treatment with pure water for the different periods of time given. The results were corrected for the amount of casein that escaped by decantation and also for the amount of free acid that remained adhering to the casein and the flask at the first filtration. The detailed results of the work are given below in Tables X. and XI.

The following statements summarize the results embodied in Tables X. and XI:

(1) When casein that has taken up acid is treated with water, it begins to lose acid rapidly to the surrounding water, and in one minute, under the conditions of the experiment, nearly one-half of the acid contained in the casein is extracted.

(2) The acid continues to pass from the casein into the water until a certain ratio is reached between the concentration of acid in the casein and the concentration of acid in the surrounding water, when equilibrium is established. This ratio is practically the same as the one found by treating casein



Table X.—Showing Amounts of Acid Taken Up by Casein, with Correction Factors.

A.	B.	C.	D.	E.	F.	G.	H.
No. of experiment.	Concentration of HCl in filtrate: ¹ (= cc. N/1000 HCl in 1 cc. solution).	Concentration of HCl in casein: (= cc. N/1000 HCl in 1 gram casein) = 100 (1.982 - B).	Grams of acid casein lost in decanting.	Grams of acid casein left in flask (= 1 - D).	Cc. N/1000 HCl left in acid-casein before extraction hering to acid-casein and flask. (= C × E).	Cc. solution added to acid-casein and flask.	Cc. N/1000 HCl in adherent solution (= B × G).
0	0.956 ¹	102.6	0.0164	0.9836	100.9	3.55	3.39
1	0.959	102.3	0.0074	0.9926	101.5	3.44	3.30
2	0.964	101.8	0.0165	0.9835	100.1	3.38	3.26
3	0.971	101.0	0.0100	0.9900	100.1	4.81	4.67
4	0.958	102.4	0.0080	0.9920	101.6	3.24	3.19
5	0.955	102.7	0.0143	0.9857	101.2	3.57	3.42

Table XI.—Showing Results of Extraction of Acid from Acid-Casein by Water.

I.	J.	K.	L.	M.	N.	O.	P.
No. of experiment.	Duration of contact between acid-casein and water.	Concentration of HCl in filtrate: (= cc. N/1000 HCl in 1 cc.).	Total cc. N/1000 HCl in solution (concentration × volume) vol. = $\frac{K \times 100}{(100 + G)}$.	Cc. N/1000 HCl extracted from acid-casein (= L - H).	Cc. N/1000 HCl left in casein (= F - M).	Concentration of HCl in casein (= $\frac{N}{E}$).	Conc'n't'n HCl in water (= $\frac{O}{P}$).
0	0	0.0327	3.39	0	100.9	102.6	3130.0
1	1 min.	0.4594	47.58	44.19	56.9	57.8	126.0
2	5 min.	0.5057	52.28	48.98	52.5	52.9	104.6
3	15 "	0.5170	53.46	50.20	49.9	50.7	98.1
4	1 hr.	0.5243	54.94	50.27	49.8	50.3	95.9
5	2 "	0.5232	54.12	50.93	50.7	51.1	97.8
6	5 "	0.5139	53.22	49.80	51.4	52.2	101.5

¹ The concentration of the acid used was 1.982 before contact with casein.

with acid until equilibrium is established, the final conditions of temperature, concentration and proportions being equal. Compare values of ratio in column P, Table XI., with those of Table IX. for three or more hours.

(3) It makes no difference whether all the acid is in the casein or in the water at the start; it divides itself between the two in a ratio which, for constant conditions, is constant.

(4) For illustration and comparison, we can use the results embodied in Table IX., Series 3, in which the final conditions of temperature, concentration and proportions are nearly the same as in the case of the results presented in Table XI. In both cases the ratio, $\frac{\text{concentration of acid in casein}}{\text{concentration of acid in water}}$, when reached under similar conditions, is practically the same (about 97) at the time equilibrium is established. The comparison is quite strikingly shown by the curves of Fig. III.,

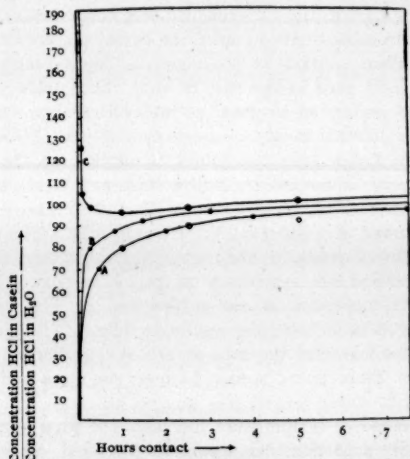


Fig. III.—Showing relation (curves A and B) of amounts of acid fixed at 25° to the concentration of acid in surrounding solution. Curve C shows rate of extraction of acid from casein that had taken up acid, and attainment of practically same equilibrium as when casein takes acid from solution (curves A and B). See Tables IX., X. and XI.

B representing the result of treating pure casein with acid and C representing the result of treating with water casein that had taken up acid.

It will be noticed that the ratio tabulated in column P, Table XI., reaches a minimum in one hour and then gradually increases. This increase is undoubtedly due to the formation of traces of dissolved casein, which, like acid globulin and albumin, as already mentioned, is doubtless a relatively weak conductor. The fixation of a very small amount of free acid in the form of soluble acid-casein would cause a noticeable decrease in the conductivity of a solution so dilute. In the second filtrate of No. 6, the xanthoproteic reaction showed traces of dissolved proteid.

Effect of Temperature upon the Action of Casein in Taking Up Hydrochloric Acid.—The effect of increase of temperature is shown by a comparison of the data contained in Tables XII. and XIII. following, with those in Tables VIII. and IX. The time of contact in the experiments was not extended beyond six hours in most cases, because the casein undergoes marked solution when contact is prolonged at higher temperatures (25° and 45°) even in the case of very dilute acid. The agitation was performed by hand at intervals and was as nearly uniform as possible in all cases, except that with N/2000 HCl at 25° the flasks were constantly in motion on the shaker.

The results embodied in Tables XII. and XIII., including temperature results also in Tables VIII. and IX., are expressed by the curves of Figs. IV., V., VI. and VII. The numbers above different points in the curves, indicating the degree of proteid solution, are explained on p. 404 in connection with Fig. I. An inspection of the tables and of Figs. IV.–VII. enables us to summarize the results as follows: (1) Increase of temperature increases the rate at which equilibrium is approached. Thus the 25° and 45° curves rise more rapidly than those for 0° .

(2) Increase of temperature increases the final amount of hydrochloric acid that disappears as free acid, *when there is marked solution of casein* due to the higher temperature, as shown by curves for 45° in Figs. IV.–VII. and for both 25° and 45°

Table XII.—Showing Amounts of Hydrochloric Acid Taken Up by Casein at 25°.

Proportions and approximate normality of acid used.	Duration of contact.	Concentration of acid in water (= cc. N/1000 acid in 1 cc. solution).	Concentration of acid in casein (= cc. N/1000 acid in 1 gram casein).	Per cent of acid in original solution taken up by casein.	Ratio of acid in 1 gram casein to acid in 1 cc. water.	Degree of solution of casein.
N/2000	See Table IX.					
N/1000	0	0.9766	0	0	0	
	15 min.	0.7330	48.7	25.0	66.5	0
200 cc.	45 "	0.6971	55.9	28.7	80.2	0
for 1 gram	1½ hrs.	0.6735	60.6	31.1	90.0	0
of casein.	3 "	0.6594	63.4	32.5	96.0	0
	6 "	0.6368	67.9	34.8	106.7	1
N/500	0	1.984	0	0	0	
	15 min.	1.569	83.0	20.9	52.9	0
200 cc.	45 "	1.332	130.4	32.8	97.8	0
for 1 gram	1½ hrs.	1.281	140.6	35.4	109.7	1
of casein.	3 "	1.262	144.4	36.4	114.4	1
	6 "	1.247	147.4	37.1	118.2	2
N/125	0	7.907	0	0	0	
	5 min.	4.079	382.8	48.4	93.9	1
100 cc.	15 "	3.901	400.6	50.6	102.7	2
for 1 gram	45 "	3.661	424.6	53.7	116.0	3
of casein.	2½ hrs.	3.509	439.8	55.6	125.4	4
	5 "	3.305	460.2	58.2	139.3	5

in Fig. VII. The soluble acid-casein apparently contains a larger proportion of hydrochloric acid than the undissolved casein, which would have the effect of raising the curve when the soluble form occurs. Our observation has been that solution of casein, whether resulting from increase of temperature, concentration of acid, or length of contact with acid, is accompanied by fixation of more acid than is taken up when no solution occurs.

(3) When rise in temperature does not result in solution of proteid, it apparently decreases the amount of acid fixed. This may be seen by comparing the value expressed in Fig. III.

Table XIII.—Showing Amounts of Hydrochloric Acid Taken Up by Casein at 45°.

Proportions and approximate normality of acid used.	Duration of contact.	Concentration of acid in water (= cc. N/1000 acid in 1 cc. solution).	Concentration of acid in casein (= cc. N/1000 acid in 1 gram casein).	Per cent of acid in original solution taken up by casein.	Ratio of acid in 1 gram casein to acid in 1 cc. water.	Degree of solution of casein.
N/2000	0	0.4980	0	0	0	
828 cc.	30 min.	0.4558	34.8	8.4	76.4	0
for 1 gram	1 hr.	0.4530	37.1	9.0	81.9	0
of casein.	4 hrs.	0.4411	47.0	11.4	106.4	0
N/1000	0	0.9928	0	0	0	
200 cc.	15 min.	0.7782	42.9	21.6	55.2	1
for 1	45 "	0.6676	65.0	32.7	97.4	2
gram of	1½ hrs.	0.6087	76.8	38.6	126.2	3
casein.	3 "	0.5808	82.4	41.4	141.9	3
	6 "	{ 0.5544	{ 87.7	{ 44.1	{ 158.1	3
		{ 0.5526	{ 88.0	{ 44.2	{ 159.3	3
N/500	0	1.984	0	0	0	
200 cc.	15 min.	1.408	115.2 (108) ¹	29.0	81.9	2
for	45 "	1.288	139.2 (106)	35.1	108.1	2
1 gram	1½ hrs.	1.230	150.8 (76)	38.0	122.6	3
of	3 "	1.171	162.6 (50)	41.0	135.6	3
casein.	6 "	1.132	170.4 (20)	42.9	154.0	4
N/125	0	7.905	0	0	0	
100 cc.	5 min.	3.540	433.5 (204) ¹	55.2	123.3	3
for 1	15 "	3.178	472.7 (—12)	59.8	148.7	5
gram of	45 "	3.080	482.7 (—160)	61.1	156.7	5+
casein.	5 hrs.	2.704	520.1 (—758)	65.8	192.8	5+

(A and B), which indicates a value of 98 to 100 for the ratio $\frac{\text{HCl in 1 gram casein}}{\text{HCl in 1 cc. water}}$ at equilibrium at 25°, with the results

in Table VIII., which indicate a value of 147 for the ratio at 0°. The same conclusion is shown by comparing the 25° and 0°

¹ Figures in parentheses give results obtained by titration method, and illustrate the effect of solution of proteid upon such results, as already shown on p. 403.

curves of Figs. IV., V. and VI., which express results obtained with acids sufficiently dilute to avoid very marked solution of proteid even at 25°. The 25° curves rise more rapidly at first than those for 0°, because the higher temperature increases the rate at which they approach equilibrium. They soon approach

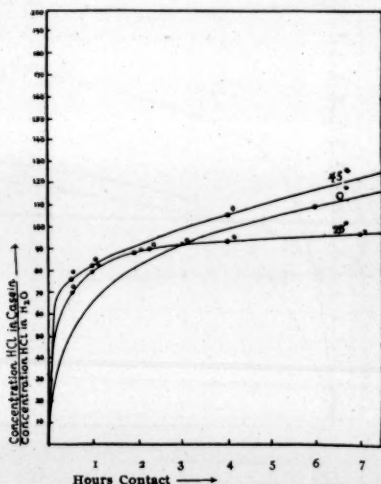


Fig. IV.—Showing effect of temperature on action of N/2000 HCl on casein. Points marked 0 signify no solution of casein detected.

a horizontal position, however, and after 2½ to 4½ hours cross the 0° curves, evidently inclining toward lower equilibrium values. With N/125 acid, however, the concentration is sufficient to dissolve decided amounts of proteid within a few

hours at 25° . Consequently, we should expect the 25° curve as well as that for 45° in Fig. VII. to be abnormally high, which is the case.

It was our purpose to study the effect of temperature upon

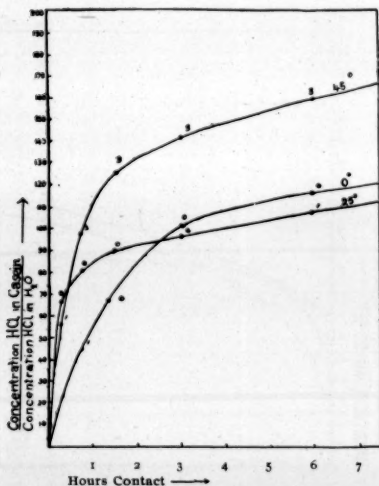


Fig. V.—Showing effect of temperature on action of N/1000 HCl on casein. The numbers 0, 1, 2, etc., represent relative degrees of solution of casein (see p. 404).

equilibrium under conditions such that solution of proteid would be more completely avoided than in the experiments given above, using only the most dilute solutions and hastening equilibrium by constant agitation in order to produce more

uniform conditions, which would appear in more regular curves. The time at our disposal for the work on casein, however, did not allow us to carry out these desired details, but the results obtained appear to be sufficiently decisive and uniform to justify the conclusions drawn.

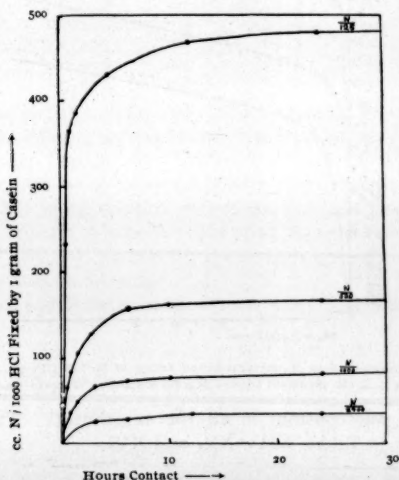


Fig. VI.—Showing effect of temperature on action of N/500 HCl on casein. The numbers 0, 1, 2, etc., represent relative degrees of solution of casein (see p. 404).

Action of Sulphuric Acid upon Casein without Solution.

The study with sulphuric acid was similar to that of hydrochloric acid, except that the effect of temperature was not investigated, while somewhat more attention was paid to the reversible character of the reaction. Because of the slighter

solvent action of sulphuric acid, the experiments could be performed at room temperature. Agitation of the reacting

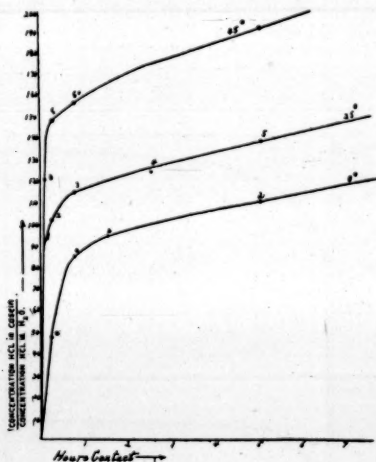


Fig. VII.—Showing effect of temperature on action of N/125 HCl on casein. The numbers 0, 1, 2, etc. represent relative degrees of solution of casein (see p. 404).

mixtures was constant in all the experiments. The results are given in Tables XIV., XV. and XVI.

Table XIV.—Showing Amounts of Sulphuric Acid Taken Up by Casein from N/125 Solution at 25° (Proportion: 100 cc. of Acid for 1 Gram of Casein).

Duration of contact.	Concentration of H ₂ SO ₄ in water (= cc. N/1000 H ₂ SO ₄ in 1 cc. solution).	Concentration of H ₂ SO ₄ in casein (= cc. N/1000 H ₂ SO ₄ in 1 gram casein).	Per cent of H ₂ SO ₄ in original solution taken up by casein.	Ratio of acid in 1 gram of casein to acid in 1 cc. water.	Degree of solution of casein.
0	8.000	0	0	0	
5 min.	3.490	451.0	56.4	129.2	0
45 "	2.022	597.8	74.7	295.6	2
3 hrs.	1.971	602.9	75.4	305.9	3
6 "	1.958	604.2	75.5	308.4	4

Table XV.—Showing Amounts of Sulphuric Acid Taken Up by Casein from N/500 Solution at 25°. (Proportion: 200 cc. of Acid for 1 Gram of Casein).

Duration of contact.	Concentration of H ₂ SO ₄ in water (= cc. N/1000 H ₂ SO ₄ in 1 cc. solution).	Concentration of H ₂ SO ₄ in casein (= cc. N/1000 H ₂ SO ₄ in 1 gram casein).	Per cent of H ₂ SO ₄ in original solution taken up by casein.	Ratio of acid in 1 gram of casein to acid in 1 cc. water.	Degree of solution of casein.
0	2.015	0	0	0	
15 min.	0.515	300.0	74.4	582	0
45 "	0.495	304.0	75.4	614	1
3 hrs.	0.493	304.4	75.5	618	1
6 "	0.489	305.2	75.7	624	2
12 "	0.491	304.8	75.6	622	2

The data in Tables XIV. and XV. are summarized as follows:

(1) Casein takes up sulphuric acid from dilute solutions more quickly than it does hydrochloric acid, the maximum amount of acid being taken up in little more than 45 minutes (Table XV.) when solution of proteid does not set in.

(2) The amount of sulphuric acid taken up by casein, the proportion of acid and casein being the same, is greater than in the case of hydrochloric acid.

(3) The equilibrium ratio is not even approximately a constant but increases rapidly with the dilution of the acid employed. This behavior is in accord with that usually observed in adsorption

Extraction of Sulphuric Acid Taken Up by Casein.—The work was carried out in detail in the manner described on p. 418. The essential results, properly corrected, are given without the details of correction in the table following:

Table XVI.—Showing Amounts of Acid Extracted from Casein by Water.

No. of experiment.	Duration of contact between acid-casein and water.	Cc. N/1000 H ₂ SO ₄ in acid-casein before extraction.	Cc. N/1000 H ₂ SO ₄ extracted from acid casein.	Cc. N/1000 H ₂ SO ₄ left in casein.	Concentration of H ₂ SO ₄ in water.	Ratio of acid in 1 gram casein to acid in 1 cc. water.
	0	301.4	0	301.4	(0.0175)	17,220
1	1 min.	301.4	32.9	268.5	0.3291	816
2	15 "	301.2	35.0	266.2	0.3489	764
3	1 hr.	301.0	37.1	263.9	0.3747	704
4	2½ hrs.	301.2	38.7	262.5	0.3775	695
5	5 "	299.6	37.4	262.2	0.3704	708

As would be expected from the high value of the ratio, conc'n H_2SO_4 in casein (Table XV.), the results indicate that conc'n H_2SO_4 in water sulphuric acid is not so completely extracted from casein by water as is hydrochloric acid. About 80 per cent of the acid that can be extracted, however, by one portion of water is extracted in one minute. After 5 hours of constant agitation with the same portion of water, only about 12 per cent of the acid that had been taken up by the casein was removed, and 1 gram of casein then contained about 700 times as much acid as did each cubic centimeter of the surrounding solution.

The tenacity with which sulphuric acid is held by casein is interesting as explaining the difficulty which Hammarsten¹ found in trying to remove sulphuric acid from casein by rubbing in a mortar with successive portions of water. Van Slyke and Hart² found that it required 85 or 90 triturations with successive portions of fresh water to remove completely the sulphuric acid from casein. It also appears from the readiness with which a given amount of water extracts the maximum amount of acid by simply shaking with the acid-casein, that a few minutes of agitation with each of a number of successive portions of pure water should remove the acid as well as trituration.

From the varying levels approached by the curves of Fig. VIII it is apparent that the equilibrium ratio, $\frac{\text{acid in 1 gram casein,}}{\text{acid in 1 cc. water}}$ is, as already mentioned above, not a constant even within the limits of concentration tested, but increases with the dilution. In order to follow the variation of the ratio, the following experiments were performed: 0.4795 gram of casein was shaken for two and one-half hours with 100 cc. of N/250 H_2SO_4 . As indicated by Table XV. and the curves, this time is sufficient for equilibrium to become practically complete and not long enough to result in appreciable solution of proteid. The suspension was allowed to settle until the supernatant solution was perfectly clear, then 50 cc. of the latter drawn off with a

¹ Maly: *Jahres d. Thierchem.*, **7**, 160 (1877).

² *THIS JOURNAL*, **33**, 469 (1905).

pipette and replaced by an equal volume of conductivity water. The mixture was then shaken two hours and 50 cc. of the solution again replaced by conductivity water; and this process was repeated twice more. The 50 cc. portions drawn off were first tested for conductivity, then titrated with N/100 KOH. In no case was any precipitate noticed on titrating, and the titration results, considering the dilution of the solution,

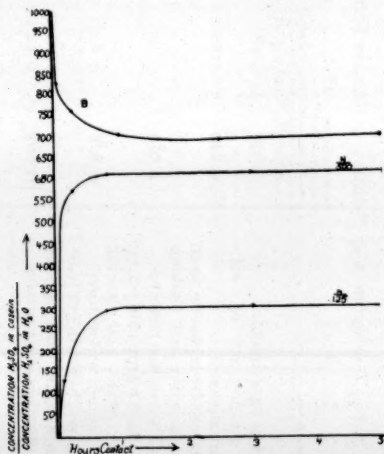


Fig. VIII.—Upper curve (B) represents action of water in extracting sulphuric acid from casein that had taken up the acid (Table XVI.). The two lower curves represent action of casein in taking up acid from N/500 and N/125 solutions of sulphuric acid (Tables XIV., and XV.).

agree throughout satisfactorily with those obtained by conductivity. Evidently no appreciable solution of proteid occurred.

It is evident that the value of the ratio varies inversely as the concentration of the acid; it increases with dilution. This increase is similar to that observed in most cases of adsorption

Table XVII.—Showing Effect of Concentration of Sulphuric Acid upon Value of Equilibrium Ratio.

No.	Description.	Concentration of acid in water (= cc. N/1000 acid in 1 cc. solution) by conductivity.	Concentration of acid in casein (= cc. N/1000 acid in 1 gr'm casein), by titration.	Ratio of acid in 1 gram casein to acid in 1 cc. water.
1	Acid as used in experiment.	4.090	4.08	0
2	Acid after shaking 100 cc. with 0.4795 gram casein.	1.547	1.60	329.4
3	Solution obtained by replacing 50 cc No. 2 with water and shaking until equilibrium was restored.	1.083	1.10	411.0
4	Solution obtained by replacing 50 cc No. 3 with water and shaking until equilibrium was restored.	0.828	0.82	465.5
5	Solution obtained by replacing 50 cc. No. 4 with water and shaking until equilibrium was restored.	0.667	0.64	498.7

phenomena and, moreover, occurs in accordance with the exponential formula, $\beta = \frac{c_1^p}{c_2}$, found empirically to hold in a large proportion of adsorptions,¹ in which β = a constant; c_1 = amount of solute adsorbed per unit mass of adsorbent = concentration of sulphuric acid in casein; c_2 = concentration of solute in solution in contact with adsorbent = concentration of sulphuric acid in water; p , the exponent of c_1 is a constant dependent like β upon the substances and conditions; $p = 1.95$

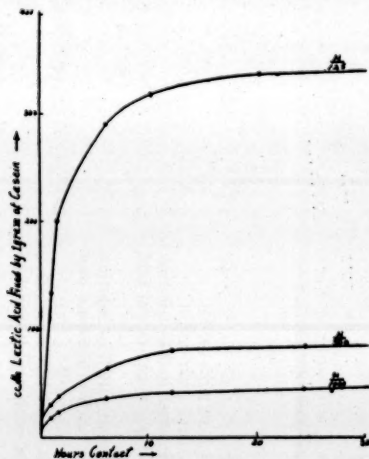


Fig. IX.—Showing (1), amounts of lactic acid taken up at 0° by casein from solutions of different concentrations, and (2) rate of reaction.

in present case. In the following table are arranged all the equilibrium results recorded in the foregoing tables. They show the regular increase of the ratio with the dilution of sulphuric acid, and the approximate constancy of β independent of the manner in which equilibrium was obtained.

¹ Schmidt: *Z. physik. Chem.*, **18**, 56 (1894). Walker and Appleyard: *J. Chem. Soc.*, **90**, 1334 (1896). Freundlich: *Z. physik. Chem.*, **57**, 385 (1906).

Table XVIII.—Showing Effect of Dilution of Sulphuric Acid upon Equilibrium Ratio, $\frac{c_1}{c_2}$.

No.	Method of obtaining equilibrium.	Tables from which results are taken.	Dilution (equivalent volume) of acid at equilibrium.	$\frac{c_1}{c_2} = \frac{\text{conc. acid in casein}}{\text{conc. acid in water}}$	$\beta \times 10^{-4} = \left(\frac{c_2}{c_1} \right)^{1.95}$
1	Shaking N/125 acid with casein.	XIV.	510.6	308.4	13.5
2	Shaking N/250 acid with casein.	XVII.	646.5	329.7	12.3
3	Replacing with water half of solution 2 in contact with acid-casein and shaking.	"	923.4	411.0	13.4
4	Replacing with water half of solution 3 in contact with acid-casein and shaking.	"	1205.0	465.5	13.3
5	Replacing with water half of solution 4 in contact with acid-casein and shaking.	"	1499.0	498.7	12.4
6	Shaking N/500 acid with casein.	XV.	2053.0	622.0	14.1
7	Shaking acid-casein with pure water.	XVI.	2703.0	695.0	13.7

Action of Lactic Acid upon Casein without Solution.

The work with lactic acid covers the concentrations, N/125, N/500, and N/1000. We used 100 cc. of each solution for 1 gram of casein. The method of experiment is that described on pp. 389-393. The results of the work are presented in Table XIX.

Table XIX.—Showing Amounts of Lactic Acid Taken Up by Casein at 0°.

Proportions and approximate normality of acid used.	Duration of contact.	Concentration of acid in water (= cc. N/1000 acid in 1 cc. solution).	Concentration of acid in casein (= cc. N/1000 acid in 1 gram casein).	Per cent of lactic acid in original solution taken up by casein.	Ratio of acid in 1 gram casein to acid in 1 cc. water.	Degree of solution of casein.
N/1000 100 cc. for 1 gram of casein.	0	0.933	0	0	0	
	5 min.	0.843	9.0	9.7	10.7	0
	45 "	0.744	18.9	20.3	25.5	0
	1½ hrs.	0.699	23.4	25.1	33.5	0
	6 "	0.586	34.7	37.2	59.2	1
	0	0.944	0	0	0	
	12 hrs.	0.532	41.2	43.6	77.5	1
	24 "	0.520	42.4	44.9	82.0	1
	48 "	0.516	42.8	45.3	83.0	2
	0	1.922	0	0	0	
N/500 100 cc. for 1 gram of casein.	5 min.	1.855	6.7	3.5	3.6	0
	45 "	1.611	31.1	16.8	19.3	0
	1½ "	1.547	37.5	19.5	24.2	0
	6 "	1.294	62.8	32.6	48.5	1
	0	1.908	0	0	0	
	12 "	1.095	81.3	42.6	74.2	1
	24 "	1.069	83.9	44.0	78.5	2
	48 "	1.062	84.6	44.3	79.7	2
	0	8.048	0	0	0	
	5 min.	7.073	97.5	12.1	13.8	1
N/125 100 cc. for 1 gram of casein.	45 "	6.723	132.5	16.5	19.7	1
	1½ hrs.	6.040	200.8	25.0	33.2	2
	6 "	5.140	290.8	36.1	56.6	3
	10 "	4.841	320.7	40.0	66.2	4
	24 "	4.652	339.6	42.2	73.0	4
	30 "	4.580	346.8	43.0	75.7	5

The results embodied in the table preceding may be summarized as follows:

(1) The amount of lactic acid taken up by casein varies with the time of contact and the concentration of the acid in which the action takes place.

(2) The amount of lactic acid taken up at equilibrium by 1 gram of casein varies nearly in proportion to the final concentration of the free acid surrounding the casein, as we have found to be true in case of hydrochloric acid. The amount of lactic acid taken up under similar conditions by 1 gram of casein is less than in the case of hydrochloric acid.

(3) In the case of lactic acid, the ratio obtained by dividing the amount of acid in 1 gram of casein by the amount of acid in 1 cc. of the surrounding solution varies from 75.7 to 83 within the concentration tested when equilibrium is approached, which occurs at 0° in 24 hours, while in the case of hydrochloric acid the ratio is about 147.

Action of Acetic Acid upon Casein without Solution.

The work with acetic acid includes experiments at 0° with N/500 and N/1000 solutions and at 25° with N/125 acid. The results are tabulated below (p. 437).

Briefly summarized, the results embodied in the following table indicate that:

(1) The action of acetic acid with casein is in a general way similar to that of the other acids previously considered.

(2) Acetic acid is taken up by casein in considerably smaller amounts than are the other acids, conditions being uniform.

(3) A comparison of the action of casein with the acids studied is graphically shown in Fig. X. The curves represent results obtained with N/500 solution in each case at 0°.

Non-Action of Casein on Dilute Solutions of Neutral Salts.

In order to determine the effect of displacing the H^+ ion from solution, half-gram samples of casein were agitated with 50 cc. portions of the pure N/50 KCl used for standardizing the conductivity cells, the duration of shaking varying from 5 minutes to 28 hours. In no case was change in conductivity observed.

Table XX. Showing Amounts of Acetic Acid Taken Up by Casein.

Proportions and approximate normality of acid used.	Temperature.	Duration of contact.	Concentration of acid in water (= cc. N/1000 acid in 1 cc. solution).	Concentration of acid in casein (= cc. N/1000 acid in 1 gram casein).	Per cent of lactic acid in original solution taken up by casein.	Ratio of acid in 1 gram casein to acid in 1 cc. water.	Degree of solution of casein.
N/500	0°	0	1.916	0	0	0	
100 cc. for	"	6 hrs.	1.430	48.6	25.4	34.0	0
1 gram of	"	12 hrs.	1.433	48.3	25.2	33.7	0
casein.	"	24 "	1.420	49.6	25.9	34.9	0
N/1000	0°	0	0.990	0	0	0	
100 cc. for	"	6 hrs.	0.749	24.1	24.3	32.2	0
1 gram of	"	12 "	0.774 ¹	21.6	21.9	27.9	0
casein.	"	24 "	0.731	25.9	26.2	36.2	0
N/125	25°	0	8.000	0	0	0	
100 cc.	"	5 min.	6.805	119.5	14.9	17.6	0
for 1 gram	"	45 min.	6.214	178.6	22.3	28.7	1
of casein.	"	3 hrs.	5.923	207.7	26.0	35.1	1
	"	6 "	5.677	232.3	29.0	40.9	2

In the case of N/125 MgSO_4 , similarly treated, the conductivity decreased only a few tenths of one per cent. From these experiments, it is apparent that the H^+ ion is the factor in solution on which depends the fixation of the large amounts of electrolyte previously determined in the experiments with the mineral acids.

DISCUSSION OF RESULTS.

In the preceding pages, it has been shown (1) that when casein and a dilute acid are shaken together, the final amount of acid taken up by a gram of casein without solution of the proteid is not constant but varies continuously with the concentration of the surrounding acid solution; (2) that, however small the amount of acid used, it is never completely taken up; (3) that the amount of acid taken up varies also with the tempera-

¹ Evidently contamination of filtrate caused abnormally high results. Low conductivity of acetic acid renders dilute solutions very susceptible to change in conductivity by slight contamination.

ture; and (4) that the acid passes as readily from the casein into the surrounding solution as from the solution into the casein, moving either way according to the change required to establish the equilibrium ratio,

$$\frac{\text{concentration of acid in casein}}{\text{concentration of acid in water}},$$

as shown in the cases of hydrochloric and sulphuric acids. It

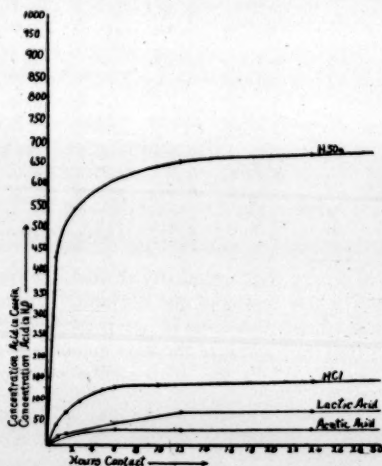


Fig. X.—Showing comparison of different acids of N/500 concentration in respect to their action in being taken up by casein at 0°.

is evident that no acid-casein compound of constant composition is formed. Three different explanations may be offered to account for the facts observed:

(1) The insoluble acid-casein may be regarded as a hydrolyzible salt, its varying composition being due to hydrolysis, which varies with the temperature and concentration of the acid.

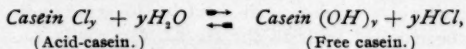
(2) Applying Witte's explanation of the fixing by fibers of substantive dyes, the reaction might be one of solution, the acid dividing itself between casein and water as resorcinol divides itself between ether and water, according to its solubility in each.

(3) The reaction may be regarded as one of adsorption, the insoluble acid-casein being what van Bemmelen calls an adsorption compound.

We will consider each of these three hypotheses in their relation to the facts that have been established.

1. *Is the Acid-Casein Solid a Hydrolyzible Salt?*

In case the insoluble acid-casein were a hydrolyzible salt of an acid, as hydrochloric acid, casein acting as a weak base, we should observe the same qualitative characteristics as those noted, *viz.*, the dependence of the amount of acid taken up by casein upon the concentration used and upon the temperature, and a loss of acid by the insoluble acid-casein when shaken with pure water. We should, however, expect somewhat more HCl than H_2SO_4 reckoned in equivalents, to be taken from solutions of equal concentration, since HCl is somewhat the stronger acid. Instead, the amount of H_2SO_4 taken up is much the greater. Furthermore, according to the law of mass action and the behavior of chemical reactions of this class, equilibrium would always be reached when the concentration of the free acid had been reduced to a certain constant value, the amount of acid held by casein being no factor in the equilibrium so long as all the basic valences of the latter were not neutralized. According to the law of mass action, if we regard the insoluble acid-casein as a salt of an acid, say hydrochloric acid for example, hydrolyzing in accordance with the hypothetical equation,



equilibrium must be reached when

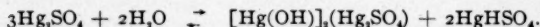
$$\frac{C_{HCl} \times C_{\text{casein (OH)}_y}}{C_{H_2O} \times C_{\text{casein Cl}_y}} = K,$$

in which C_{HCl} = concentration of HCl; $C_{\text{casein (OH)}_y}$ = concentration of free casein, etc.; y = basic valence of casein; K_t = a constant dependent on temperature. The hydrochloric acid is the only reagent of variable concentration. Consequently,¹ the equation simplifies to

$$C_{HCl} = K_t$$

The accuracy of this form of equation for reactions involving only one substance of variable concentration has been repeatedly proved by well-known reactions. In the decomposition of CaCO_3 by heat, $C_{\text{CO}_2} = K_t$. In the reaction by which ammonia and CaCl_2 combine, the pressure (concentration) of ammonia at equilibrium depends only on the temperature. The same is true of the reaction between PbO and NH_4Cl , in which ammonia is formed.²

As examples in which the reaction is hydrolysis and therefore especially comparable with the hypothetical hydrolysis of insoluble acid-casein, the hydrolysis of mercurous sulphate and of the picrate of diphenylamine may be noted. The first reaction was studied by Gouy³ and by Hulett.⁴ It occurs in accordance with the equation:



The HgHSO_4 is relatively the only soluble substance and consequently the only one of varying concentration. Equilibrium is reached when C_{HgHSO_4} = constant, this constant concentration at 25° being 0.00225 molecular weights in grams per liter. When Hg_2SO_4 , or a mixture of it with the basic salt, was shaken with water, hydrolysis always proceeded until the concentration of the HgHSO_4 reached this value, and no further hydrolysis occurred unless the mixture of salts was treated with fresh water, when the process again went to the same limit, and it could be repeated until all the Hg_2SO_4 was changed. Hulett treated a portion of Hg_2SO_4 thus with forty-three successive

¹ "Only gaseous and dissolved substances have varying concentrations." Ostwald: "Principles of Inorganic Chemistry," translation by Pindlay, p. 327 (1902).

² Isambert: *Compt. rend.*, **102**, 1313 (1886).

³ *Compt. rend.*, **130**, 1399 (1900).

⁴ *Z. physik. Chem.*, **49**, 491 (1904).

portions of water and found at the end of each treatment (of 12 hours) the same concentration of HgHSO_4 in solution. The forty-third treatment exhausted the Hg_2SO_4 , and further treatment had no effect except to dissolve traces of the basic salt.

The picrate of diphenylamine, like all salts of this base,¹ is hydrolyzed by contact with water, with formation of picric acid and diphenylamine. Both diphenylamine and its picrate are, like casein and acid-casein, practically insoluble. The picric acid is, therefore, the only reagent of variable concentration. Walker² found that picric acid (saturated solution) placed in contact with varying excess portions of diphenylamine always combined with it until the concentration of the acid was reduced to 13.8 mg. per cubic centimeter. If picric acid of less concentration than 13.8 mg. per cubic centimeter was placed in contact with diphenylamine no combination occurred.

From the preceding discussion of adsorption reactions and of hydrolysis in which, of the reacting substances, only the acid has variable concentration (that is, is soluble), it is evident that the nature of such a reaction is represented by the curves of Fig. XI.

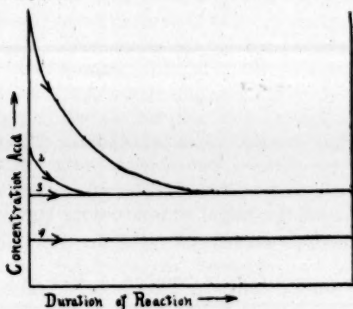


Fig. XI.

¹ Meyer und Jacobson: "Lehrbuch der Organischen Chemie," Vol. II. [1], p. 177 (1902).

² J. Chem. Soc., 69, 1341 (1896).

The four curves in this diagram represent the theoretical effect of contact with an excess of insoluble base upon portions of acid of varying concentration, the conditions, other than concentration of acid, being the same for the four cases represented in each figure.

The diagrammatic curves of Fig. XI. signify that:

(1) When an insoluble base, forming an insoluble hydrolyzable salt, is treated with acid of the equilibrium concentration (curve 3), as, for example, 13.8 mg. per cubic centimeter in the case of picric acid and diphenylamine, none of the acid is combined, so that its concentration remains constant.

(2) Likewise, when the initial concentration is less than that required for equilibrium, none is combined (curve 4).

(3) When the initial concentration is greater than that required for equilibrium (curves 1 and 2), acid is combined until the concentration of free acid is reduced to the equilibrium value, which is the same, whatever the initial concentration of the acid.

The curves of Fig. XII. express results obtained with four initial concentrations of hydrochloric acid, as given on p. 415 in Table VIII., column 3. Similar curves are obtained by plotting the results tabulated for the other acids. The forms of the curves (compare with Fig. XI.) show clearly that the reaction does not fulfil the requirements characteristic of the formation of a hydrolyzable salt, but, as will be shown later, possesses the characteristics of an adsorption. The final concentration is not a constant, but varies with the initial concentration in all cases (note different levels approached by curves of Fig. XII.), and the initial concentration, however small, is always reduced by contact with casein.

2. Is the Reaction a Case of Solution of Acid in Casein?

Most of the qualitative characteristics of the reaction, such as the lack of definite chemical combining proportions and the ability of the acid to divide itself according to a definite ratio between casein and water, are characteristics of the partition of a solute between two solvents as well as of adsorption. The behavior

of the ratio $\frac{c_1}{c_2}$ in the case in question, particularly when sulphuric acid is considered, does not, however, correspond to the conditions of distribution in such cases.

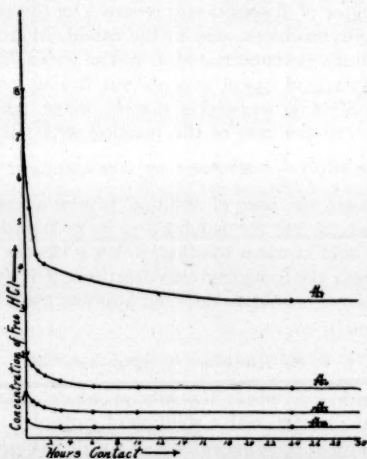


Fig. XII.—Showing decrease of concentration of free hydrochloric acid resulting from action of casein in taking up acid (Table VIII., column 3).

To quote Walker in regard to the law of distribution:¹ "If the molecular weight of the substance in one solvent is n times as great as its molecular weight in the other solvent, then, when equilibrium is attained, the n th root of the concentration in the first solvent will bear a constant ratio to the concentration in the second solvent." According to this, the molecular weight of hydrochloric acid would have to be regarded

¹ J. Chem. Soc., 69, 1335 (1896).

as the same in water that it is in its hypothetical solution in casein, in order that the ratio, $\frac{\text{concentration of acid in casein}}{\text{concentration of acid in water}}$, should be constant. As is shown, however, by the freezing point, osmotic pressure and electrical conductivity of hydrochloric acid solutions, the hydrochloric acid molecule is almost completely dissociated in water at the concentrations employed. An equal degree of dissociation is required by the laws of solution for the hydrochloric acid in the casein, where it is from 100 to 150 times as concentrated as in the water. This would require us to regard casein as a solvent having a remarkable dissociating effect far surpassing that of water, which is very improbable. In the case of the reaction with sulphuric acid in which the ratio $\frac{c_1}{c_2}$ decreases as concentration of acid in water increases, the laws of solution require a much smaller molecular weight for the sulphuric acid in casein than that of sulphuric acid in water solution, which is clearly an impossibility. From the foregoing considerations, it is evident that the hypothesis of a solution does not apply to the action under consideration.

3. Is the Reaction One of Adsorption?

By adsorption is meant the process whereby a solid substance, A, in contact with a solution of a dissolved substance, B, concentrates B upon its surface or within the meshes of its physical structure, withdrawing a portion of B from solution, without forming a definite chemical compound with it. In regard to the characteristics of adsorption reactions, we quote the five criteria given by van Bemmelen for reactions of this class.¹

"(1) The adsorbed amounts stand in no equivalent proportions to the adsorbing mass.

"(2) The composition varies with the structure of the adsorbing substance and with all modifications which the latter undergoes by reason of its method of preparation, age, heat or action of other substances.

¹ Z. anorg. Chem., **26**, 381 (1903).

"(3) The composition varies with the temperature.

"(4) Likewise with the concentration of the solution, in case substances are adsorbed from their solutions; and the composition varies in such manner that the adsorption factor k in the formula $\frac{c_1}{c_2} = F(k)$ is not constant, but is dependent upon the concentration of the solution according to a function that we do not understand. In general, however, c_1 increases more slowly than c_2 " ($\frac{c_1}{c_2}$ decreases as concentration increases).

c_1 = amount of solute adsorbed per unit mass of adsorbent.

c_2 = concentration of solute in solution.

F = unknown function.

In another article,¹ however, van Bemmelen says: "When the adsorptive power is small and the solution not concentrated, then, between certain limits of concentration, $\frac{c_1}{c_2}$ is nearly constant."

In regard to the variation of $\frac{c_1}{c_2}$, while, as van Bemmelen states, an entirely general and accurate formula is unknown, the relation in a large proportion of adsorptions can be expressed with a fair degree of approximation by the equation, $\beta = \frac{c_1^p}{c_2}$, β and p being constants dependent in value upon the active substances and the conditions.² The formula has been found to hold by Freundlich, Walter and Appleyard, and Schmidt.³ In the experiments of Schmidt, p was found to vary from 2 to 10, while in those of Freundlich³ p did not vary greatly from 2 in most cases. When p becomes unity, the formula applies to the case in which $\frac{c_1}{c_2} = \text{constant}$.

(5) "The velocity with which the formation of an adsorption compound occurs decreases continually as more substance is adsorbed and the adsorption approaches equilibrium." In

¹ Z. physik. Chem., 18, 33 (1895).

² Loc. cit.

³ Loc. cit., p. 396.

another article van Bemmelen¹ says: "The adsorption begins with great speed and decreases continually as more substance is adsorbed when equilibrium is approached. With constant shaking, the latter is usually reached in one to three hours."

To the above we may add the two points following:

(6) The reaction approaches a true equilibrium, which can be approached from either side, as stated by Ostwald² and as shown by the work of Freundlich³ and also by Walker and Appleyard.

(7) Ostwald⁴ expresses graphically as follows the characteristic relation between the concentration of the solution and amount of solute adsorbed. Using rectangular co-ordinates, if one plots dilution of the solution as ordinates and amounts of solute adsorbed as abscissae, the resulting curve will be nearly a hyperbola.

Of the above, the first and most important point corresponds entirely with the facts observed in the reaction between casein and acids. There are no definite combining proportions.

None of our data bears upon the second point, since the casein was all prepared and kept by one uniform method.

The third statement is in harmony with our results. In the cases tested, temperature was found to have a considerable influence upon the amount of acid fixed.

Regarding the fourth point, it was found that in all cases the fundamental principle was complied with, that is, the amounts of acid taken up per gram of casein at equilibrium depend upon the final concentrations of the solutions. In the cases of hydrochloric, lactic and acetic acids, $\frac{c_1}{c_2} = \left(\frac{\text{concentration of acid in casein}}{\text{concentration of acid in water}} \right)$ is nearly constant. This, according to van Bemmelen, may occur when solutions are dilute and adsorptive power weak. While the adsorptive power, particularly in the case of hydrochloric acid, is not weak, the solutions were very dilute. In the case of sulphuric acid, where

¹ Z. anorg. Chem., **23**, 345 (1900).

² Lehrb. d. Allg. Chem., 2d Aufl., **1**, 1093.

³ Loc. cit., p. 389.

⁴ Loc. cit.

adsorption is much stronger, the ratio $\frac{c_1}{c_2}$ increases with the dilution in accordance with the exponential formula $\beta = \frac{c_1^p}{c_2}$, which has been found to hold in a large proportion of adsorptions, p being 1.95 in this case (p. 433). If p be taken nearly equal to unity, the same formula holds for the other acids.

In velocity, the reaction, agreeing with van Bemmelen's fifth criterion, begins with great speed and becomes slow as equilibrium is approached. Particularly when the mixtures of casein and acid are subjected to constant agitation so that the casein does not have to wait for acid to reach it by diffusion, the initial velocity was enormous, the curves being nearly parallel to the ordinate axis for some distance. Negative acceleration is, of course, observed in reactions in general as they approach equilibrium, so that no great force is to be attributed to this characteristic for the purpose of classifying the reaction, although the enormous initial velocity is not usually observed in intermolecular reactions requiring considerable time for completion. The great velocity with which acid is extracted by water from the insoluble acid-casein furnishes a marked contrast to the stability of the precipitated acid albumin of von Rohrer, an apparently true chemical compound, which could be washed with water without changing materially its acid constant.

The fact that the equilibrium can be approached from both sides has been shown in the cases of both hydrochloric and sulphuric acids.

Finally, the fact that the characteristics of the reaction indicate adsorption rather than the formation of an insoluble salt, is brought out with striking clearness by the application of Ostwald's graphic criterion for adsorption. According to this, if, using equilibrium data, dilutions of the solution are plotted as ordinates and amounts of solutes adsorbed as abscissae, the characteristic curve for adsorption is similar to a hyperbola. In the case of formation of a hydrolyzible salt of the kind in question, the curve would be very different. For the combination of any acid at all, the dilution could not exceed that corre-

sponding to the concentration constant. Nor, until the base is completely neutralized, can acid in contact with it at equilibrium be of less dilution than the constant, since the acid will continue to combine until that dilution is reached. Consequently, as more acid is added to a solution in contact with the base, the latter takes it up, keeping the dilution constant until the basic valences are all saturated, the curve expressing these conditions being a horizontal line. Further addition of acid simply decreases the dilution, since further combination is impossible and the curve drops perpendicularly. The two types of curves are illustrated in Fig. XIII. Curves 1 and 2

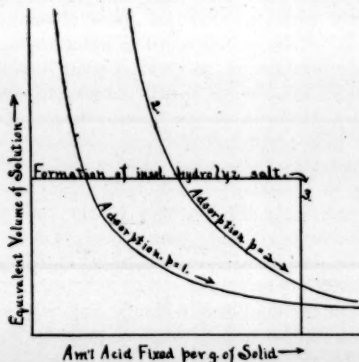


Fig. XIII.—Typical curves contrasting relations in adsorption with those in formation of an insoluble hydrolyzible salt from an insoluble base. The adsorption-curves are plotted in accordance with the exponential formula $\beta = \frac{C_1^p}{C_2}$. Arrows above curves indicate direction of change caused by addition of acid.

represent adsorption, following the equation, $\beta = \frac{C_1^p}{C_2}$, in which p has the values of 1 and 2 respectively. In the former case, the curve is a true hyperbola; in the latter, it represents the average relations observed by Freundlich in ad-

sorption by charcoal. The rectilinear curve 3 represents the conditions in the formation of a solid salt, as described above. In Fig. XIV. the results experimentally obtained with the various acids are graphically represented. Comparison with Fig. XIII. shows that the reactions represented are adsorptions.

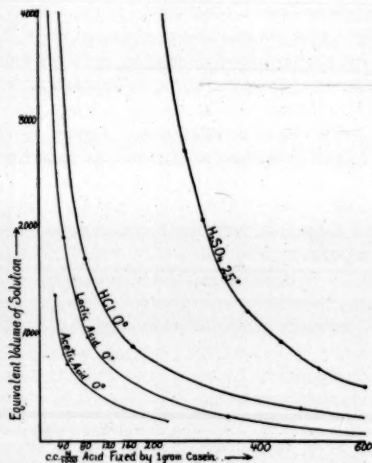


Fig. XIV.—Curves express relation at equilibrium between dilution of various acids and amounts fixed by 1 gram of casein. Comparison with Fig. XIV. shows that the curves are characteristic of adsorption. Comparison of the curves with each other at the same dilution shows the relative amounts of the different acids adsorbed.

From the results presented above, it is apparent that the reaction by which undissolved casein reduces the concentration of an acid solution is one of adsorption and not the formation of either a stable or a hydrolyzible salt, as is shown by the absence of either constant combining proportions or a constant concentration of acid at equilibrium.

Casein is not the first proteid whose adsorptive power for

acids has been noted. Walker and Appleyard¹ found that silk fixed varying proportions of mineral, fatty and aromatic acids, the aromatic being adsorbed most completely, mineral acids next, and fatty acids least; and it was shown that the reaction was one of adsorption. Schmidt² found that the process by which silk fixed the acid dye eosin was one of adsorption, and concluded from this and other data that dyeing is an adsorption phenomenon. Sjöqvist³ found that coagulated egg albumin takes hydrochloric and sulphuric acids from solutions, and that the acids could be re-extracted from the albumin with water, the reaction being characteristic of adsorption, although Sjöqvist attributes it *à priori* to the formation of a hydrolyzible albumin-acid salt. Using in each case 2 grams of albumin for 100 cc. of acid, he found that in five minutes, 72, 83 and 91 per cent of the acid was removed from solutions, respectively, of N/10, N/20 and N/40 concentration. It is apparent that, although the time of reaction was too short to permit final conclusions, the results suggest an adsorption, inasmuch as the concentration of the acid was reduced in all cases and more markedly as dilution increased, and showed no tendency towards an equilibrium constant. Hardy⁴ calls attention to the fact that the reacting masses of dissolved globulin and acid are not constant, and while favoring the idea of chemical combination in the case of globulin, he is inclined to suggest the possibility that the relation between electrolytes and proteids, even in solution, may be one of adsorption, the proteid molecules being gathered into minute masses in suspension, which form an "inner phase" more or less rich in adsorbed electrolyte. Hardy emphasizes the "need for caution in regarding the reaction between proteids and acids as one of simple salt formation. The possibility of the phenomena belonging to the intricate border-land of adsorption combination must not be lost sight of."

The selective action of casein in taking acids but not neutral mineral salts from solution is paralleled by that of charcoal.

¹ J. Chem. Soc., 69, 1334 (1896).

² Z. physik. Chem., 15, 60 (1894).

³ Skandin. Arch. Physiol., 5, 35 (1895).

⁴ Jour. Physiol., 22, 300 (1905).

Freundlich¹ finds that a carefully purified preparation of the latter adsorbs mineral and organic acids, particularly the latter, but alters the concentration of mineral salts by less than 1 per cent. In this case the adsorbent is equally inert in a chemical sense towards either acids or salts. The reaction is purely one of surface energy, independent of any possibility of chemical combination between adsorbent and dissolved substance, and the same is probably the case with casein.

It appears, however, that in all cases adsorptive power and the tendency to react chemically are not so sharply separated. Van Bemmelen² says: "If P and C (adsorbent and dissolved substance) are indifferent to each other according to the ordinary chemical conception, an adsorption may occur, but it is in many cases only a weak one." He found that the hydrogel of SiO_2 adsorbed strong bases much more strongly than strong acids or salts. Manganese dioxide, which has acid properties and can combine chemically with strong bases, adsorbs alkalis better than it does acids or salts. Metastannic acid, which can form combination with acids, adsorbs potassium chloride weakly, hydrochloric acid strongly. From these and similar facts, van Bemmelen concludes that it appears that "in many cases adsorption is distinguished as the *forerunner of chemical combination*." Adsorption is strong in many cases when, under other conditions or by gradual inner changes, chemical compounds can be formed. For example, van Bemmelen found³ that colloidal silica in contact with barium hydroxide adsorbed the latter in accordance with the criteria of adsorption, so long as barium hydroxide of less than a certain concentration was used. When this concentration was exceeded, combination occurred and a compound having the composition $\text{BaSiO}_3 \cdot 6\text{H}_2\text{O}$ separated in crystalline form. In double transposition in solution, as between salt and salt, the new compound is often precipitated as a colloid and changes later into a chemical compound of crystalline form and definite composition. It is questionable whether the colloidal precipitate first formed is a chemical compound of definite propor-

¹ *Loc. cit.*

² *Zeit. f. anorg. Chem.*, **23**, 342 (1900).

³ *Z. anorg. Chem.*, **26**, 380 (1903).

tions rather than an adsorption compound, or a transition between the latter and a definite chemical compound. Van Bemmelen has determined the transition of precipitated colloidal adsorption compounds of Al_2O_3 , BeO and CuO into the chemical compounds.

From the point of view of van Bemmelen discussed in the preceding paragraph, *viz.*, that a chemical affinity between two substances, insufficient to cause a chemical reaction under existing conditions, may result in the formation of an adsorption compound, which may or may not give rise to a definite chemical compound, the action between casein and acids, by which insoluble acid-casein with varying content of acid is formed, might be regarded as the formation of an adsorption compound under such influence; and the change to soluble acid-casein formed on long standing, on warming, or on contact with acids above a certain degree of concentration, as the change of the adsorption compound into a chemical one, such as a proteid acid salt.

Assumptions aside, however, as to the possible relation between the basicity of casein and its adsorptive power, and as to the nature of soluble acid-casein, the action of dilute acids may be briefly stated thus: The acids first concentrate upon the casein by adsorption and then begin to dissolve it more or less slowly according to conditions of temperature, concentration, etc. The dissolved substance, at least in the case of hydrochloric acid, contains more acid than the adsorption compound.

Although the results obtained during our limited time of investigation in this line of work are not complete, the facts that have been presented, viewed in the light of our present knowledge regarding reactions having similar characteristics, have forced us to regard the action studied as a case of adsorption, with the formation of what van Bemmelen calls "adsorption compounds," that is, physical combinations with composition varying continuously with the conditions. It must be confessed that much remains to be learned about adsorption compounds and the phenomena of adsorption. They have, however, certain characteristics in common, as summarized on pp. 444-445, and the possession of these characteristics by the reaction between

dilute acids and casein, together with the complete failure of the reaction to agree with the accepted laws of chemical combination, lead us to classify the process as an adsorption. Speculations as to whether other proteids also take up acids by adsorption, or whether casein and silk are peculiar in this respect, are useless until careful quantitative tests have been made with other proteids.

For an interesting speculation upon the possible biological significance of adsorption, the reader is referred to the discussion at the close of Freundlich's recent paper.¹

Viewing the action between casein and acids, as an adsorption, practically brings us back to Hammarsten's original proposition, in regarding the action as one not strictly chemical, though his conclusion was based on results that could not be accepted as at all decisive, especially in view of the facts we now possess which were not then known.

SUMMARY.

1. *Object.*—The work of which an account has been presented was undertaken primarily to measure more accurately than had been done² the amount of acid that combines with casein to form a definite compound insoluble in water, but facts were developed which raised the fundamental question as to whether the action is one of real chemical combination and to this question chief attention was directed.

2. *Outline of Method of Work.*—Casein is agitated with dilute acids of known strength for given periods of time, the mixture is filtered, and the amount of acid taken up by the casein is measured by the decrease in the conductivity of the solution. It was found necessary to work out special formulas in order to calculate conveniently the results of conductivity measurements into terms giving equivalent amounts of acid. In some cases casein that had taken up acid was treated with water and the acid partially extracted.

3. *General Scope of Work.*—The behavior of casein was studied (1) with four acids of different dissociating power

¹ Z. physik. Chem., **57**, 385 (1906).

² THIS JOURNAL, **33**, 461 (1905).

(hydrochloric, sulphuric, lactic and acetic); (2) with N/125, N/500, N/1000 and N/2000 concentrations of these acids; (3) for contact of different periods of time, ranging from 5 minutes to 48 hours; (4) at different temperatures (0° , 25° and 45°); and (5) with dilute solutions of neutral salts (potassium chloride and magnesium sulphate).

4. *Solubility of Casein in Dilute Acids.*—It was necessary first to ascertain conditions under which casein forms soluble compounds with dilute acids in order to avoid such solution. Evidence of solution was shown (1) by viscosity, (2) by behavior of solution on adding alkali, such as opalescent or milky appearance or precipitation, (3) by the xanthoproteic reaction, and (4) by deviation between results obtained by conductivity and by titration methods. In case of solution of casein, titration results are untrustworthy for measuring the amount of free acid in solution, because the dissolved proteid *per se* neutralizes part of the alkali used for titration and also gives up to the alkali any acid with which the proteid may have combined. Little or no solution of casein occurs even on several hours' contact at 0° with solutions not stronger than N/500, or at 25° with solutions not stronger than N/1000. Degree of solution is increased (a) by concentration of acid, (b) by increase of temperature and (c) by prolongation of contact. The solvent action of the four acids studied was in the following order, from strongest to weakest, hydrochloric, lactic, sulphuric and acetic. The rate at which casein dissolves in different acids of the same normality is not proportional to the concentration of the H^{+} ions, or to the dissociation, but is disproportionately great for the weak organic acids. From solution of equal strength, the dissolved proteid takes up a larger proportion of acid than does the undissolved.

5. *General Action of Acids and Casein without Solution.*—Casein takes up acids from dilute solutions. For example, 1 gram of casein, shaken with 100 cc. of N/1000 hydrochloric acid for 3 hours, takes from the solution nearly 50 per cent of the acid. The amount of acid thus taken up is not definite and fixed, but varies (1) with the concentration of the acid, (2) with the duration of contact until equilibrium is reached,

which require some hours, (3) with the degree of agitation until equilibrium is reached, (4) with the temperature, and (5) with the kind of acid. Some acid is always taken up, however small the amount of acid used, but the acid is never completely removed from the solution, however large the proportion of casein present.

6. *Equilibrium Ratio of Reaction.*—The maximum amount of acid adsorbed by a gram of casein varies almost directly with the concentration of the acid within the limits used (N/125 and N/1000) in the case of hydrochloric, lactic and acetic acids, so that at equilibrium the ratio of acid in 1 gram of casein to the acid in 1 cc. of surrounding solution is nearly constant. In the case of sulphuric acid, the ratio increases with dilution of acid. The equilibrium ratio reached, when 1 gram of casein is treated with 100 cc. of N/500 solution at 0°, is 675 for sulphuric acid, 147 for hydrochloric acid, 80 for lactic and 36 for acetic. Equilibrium is reached in 2 to 24 hours according to (1) the acid used, (2) the temperature and (3) the degree of agitation. The greater part of the acid reacting is generally taken up in the first half hour or hour. Increase in temperature increases the rate at which equilibrium is approached but decreases the final amount of acid taken up, when solution of casein does not result at the higher temperature.

7. *Extraction of Acid from Casein.*—The acid taken up by casein may be extracted by shaking with water. The extraction continues until the equilibrium ratio (concentration of acid in 1 gram of casein divided by concentration of acid in 1 cc. of surrounding solution) is established, and then ceases.

8. *Neutral Salts.*—Neutral salts (potassium chloride and magnesium sulphate) are not adsorbed from dilute solution by casein.

9. *Character of Reaction.*—The behavior of casein with dilute acids, when no solution occurs, suggests three different explanations: (1) an adsorption compound, (2) solution of acid in casein or (3) a hydrolyzible salt of casein. A careful application of the experimental results to each of these hypotheses indicates that the action appears to correspond in detail only with the characteristics which apply to an adsorption.

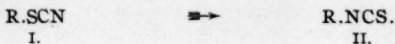
10. *Reaction Applied to Precipitation of Casein in Saving of Milk.*—On the basis of this explanation, the proteid precipitated when milk sours is free casein in which lactic acid is held by adsorption.

CXLVII. — RESEARCHES ON THIOCYANATES AND ISOTHIOCYANATES: DIPHENYLCARBAMYLTHIOCYANATE.

SEVENTH PAPER.

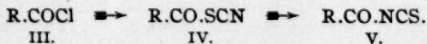
BY TREAT B. JOHNSON AND L. H. LEVY.

In every case that has been carefully examined a thiocyanate, I., is the primary product of the reaction when an alkyl halide is treated with ammonium or potassium thiocyanate. Whenever an isothiocyanate, II., is obtained it results from the molecular rearrangement of the isomeric thiocyanate I.



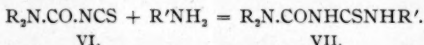
In the case of acyl halides, III., the products of the reaction have always been assigned the isothiocyanate structure, V., and those investigators¹ who have studied this reaction have never obtained any positive evidence of the formation of two distinct isomers.

We shall describe in this paper a case where a normal acyl thiocyanate, IV., is obtained by treatment of an acyl chloride, III., with potassium thiocyanate. Our observation seems to indicate that acyl halides, III., in general, react in the same manner as alkyl halides with potassium thiocyanate to form thiocyanates, IV., which then undergo a metamer change to isothiocyanates, V.

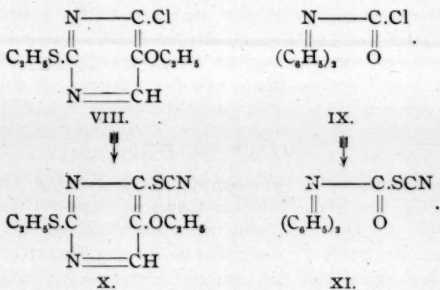


¹ Miquel: *Ann. Chim. Phys.*, [5], 11, 318 (1877). Dixon: *J. Chem. Soc.*, 67, 363, 1040; 69, 855, 1593; 71, 617; 73, 388; 75, 541; 83, 84; 85, 807; 87, 331; 89, 892. Dixon and Hawthorne: *Ibid.*, 87, 468. Doran: *Ibid.*, 89, 328; 79, 906.

Dixon¹ has investigated the behavior of diphenyl-, methylphenyl-, ethylphenyl- and benzylphenylcarbamyl chlorides towards inorganic thiocyanates. He observed that they reacted to form isothiocyanates. In no case, however, was the isothiocyanate, VI., isolated in a state of purity, but their formation was established by the fact that thiobiurets, VII., were obtained when the crude reaction products were treated with organic bases:



In a previous paper from this laboratory Johnson and McCollum² have shown that 2-ethylmercapto-5-ethoxy-6-chloropyrimidine, VIII., gives a thiocyanate, X., when treated in alcohol with potassium thiocyanate. On account of the close analogy of diphenylcarbamyl chloride, IX., to this cyclic imide chloride, VIII., and its stability in warm alcohol, it appeared to the writer that the carbamyl chloride, IX., might react with potassium thiocyanate under similar conditions to give a normal acyl thiocyanate, XI.



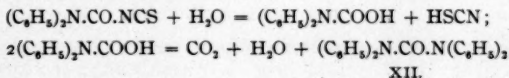
Our experiments have confirmed this assumption. We find that diphenylcarbamyl chloride, IX., reacts smoothly with potassium thiocyanate to give diphenylcarbamyl thiocya-

¹ J. Chem. Soc., 75, 388.

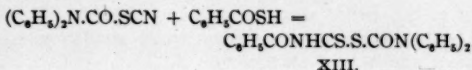
² THIS JOURNAL, 36, 136 (1906).

nate, XI., the first representative of the hitherto unknown series of acyl thiocyanates, IV. That a thiocyanate is the first product of the reaction, we have established in the following manner: It showed no tendency to react with organic bases, and underwent no change when digested with hydrochloric acid.

Dixon¹ has shown that the isomeric isothiocyanate is decomposed by steam distillation with formation of thiocyanic acid, carbon dioxide, water and tetraphenylurea, XII., according to the following equations:



Our compound reacted with thiobenzoic acid² as a normal thiocyanate giving a dithiourethane, XIII. We did not observe any formation of carbon bisulphide:



The urethane structure was confirmed by the yellow color of the compound and its solubility in alkali.

It seems very probable to the writer that all the carbamyl chlorides examined by Dixon will give thiocyanates if treated with potassium thiocyanate under the proper conditions.

EXPERIMENTAL PART.

Diphenylcarbamyl Thiocyanate, $(\text{C}_6\text{H}_5)_2\text{N.CO.SCN}$.—This compound was prepared in the following manner: 20 grams of diphenylcarbamyl chloride were first dissolved in 50 cc. of 95 per cent alcohol. Ten grams of dry potassium thiocyanate were then added and the solution heated on the steam-bath for 3–4 hours. The insoluble potassium chloride was then filtered off and the alcoholic solution allowed to cool. The thiocyanate deposited in prismatic crystals, which were purified for analysis by recrystallization from alcohol. It melted

¹ *Loc. cit.*

² *J. Am. Chem. Soc.*, **23**, 283.

at 138° to a clear oil. The thiocyanate was also prepared by digesting the carbamyl chloride in acetone with potassium thiocyanate. Analysis (Kjeldahl):

	Calculated for $C_{14}H_{10}ON_2S$	I.	Found.	II.
N	11.02	10.97		11.00

Action of Hydrochloric Acid.—One gram of the thiocyanate was heated with 10 cc. of concentrated hydrochloric acid for two hours. It was not altered by this treatment and melted at 138° .

Action of Ammonia and Aniline.—The thiocyanate showed no tendency to react with bases. It was recovered unaltered after suspension in concentrated ammonia for twelve hours. Half a gram of the thiocyanate was mixed with aniline and allowed to stand at ordinary temperature. There was no evidence of any reaction after standing for six weeks.

Action of Thiobenzoic Acid.—Five grams of the thiocyanate and 2.7 grams of thiobenzoic acid were heated together in a water bath. There was no apparent reaction at first but at 70° a gas began to be evolved and at 90° a violent reaction took place. The heating was continued at $90-100^{\circ}$ until the evolution of gas practically ceased, when approximately 350 to 400 cc. of gas had collected over water. It burned with a blue flame and emitted a strong odor of sulphur dioxide. It was identified as carbon oxysulphide. We obtained no evidence of the presence of carbon bisulphide. The residue was a thick varnish that showed no signs of solidifying after standing for several hours. It was treated with a solution of sodium hydroxide and the insoluble portion removed by extraction with ether (see below). When the alkaline solution was saturated with carbon dioxide, a canary-yellow precipitate was obtained that crystallized from hot benzene in prisms and melted at $128^{\circ}-129^{\circ}$ to a clear oil. The compound was very soluble in alcohol and volatilized with alcohol vapors when its solution was evaporated on the steam bath. A nitrogen determination (Kjeldahl) agreed with the calculated value in benzoaldithiodiphenylcarbamyl carbamate, $C_6H_5CONHCS.S.CON(C_6H_5)_2$.

	Calculated for $C_{21}H_{18}O_2N_2S_2$.	Found.
N	7.14	7.06

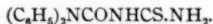
The ether washings from the above treatment with sodium hydroxide deposited a crystalline compound. It was purified for analysis by recrystallization from alcohol and separated in well developed, prismatic crystals that melted at 177° . They did not respond to a test for sulphur and were insoluble in alkalis and acids. The compound was identified as *benzoyldiphenylamine*, $C_6H_5CO.N(C_6H_5)_2$. Analysis (Kjeldahl):

	Calculated for $C_{13}H_{10}ON$.	Found.
N	5.12	5.27

Action of Thioacetic Acid.—The thiocyanate showed less tendency to react with thioacetic acid than with thiobenzoic acid. By careful treatment the thiocyanate could be repeatedly recrystallized from the thioacid and be recovered unaltered. On prolonged heating at 80° – 90° there was a slow evolution of gas, and a thick varnish was obtained that showed no signs of solidifying after standing for several days. It was not examined further.

Behavior of the Thiocyanate on Heating.—The thiocyanate apparently shows no tendency to rearrange into its isomer at ordinary temperature. It was also preserved at a temperature of 60° – 80° for several weeks without change. One gram of the thiocyanate was heated in an oil bath for an hour at 138° – 141° . We obtained a thick varnish that would not solidify on cooling. It had a disagreeable odor and slowly decomposed when heated above 160° . That this was an isothiocyanate derivative was established by its behavior with bases. It reacted at once with aniline to form a,c,c-triphenylthiobiuret melting at 167° – 168° . Dixon states that this compound melts at 163° – 163.5° . In another experiment 2 grams of the thiocyanate were rearranged to the isothiocyanate by heating for one hour at 150° – 160° . The isothiocyanate was then treated with concentrated aqueous ammonia and allowed to stand. We obtained a crystalline compound that crystallized from

alcohol in prisms. The melting point, 182° – 183° , agreed with that given by Dixon for *diphenylthiobiuret*,



Analysis (Kjeldahl):

N	Calculated for $\text{C}_{14}\text{H}_{13}\text{ON}_3\text{S}$.	I.	Found.	II.
	15.49	15.54		15.52

NEW HAVEN, CONN.,
June 20, 1907.

Contributions from the Chemical Laboratory of Harvard College.

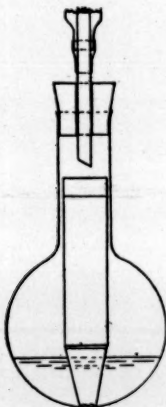
AN EXTRACTOR FOR USE WITH SMALL QUANTITIES OF MATERIAL.

BY C. LORING JACKSON AND J. E. ZANETTI.

This form of extractor was contrived by one of us more than twenty years ago, and has been in use at intervals in this laboratory ever since. It is so simple that it seems as if it must have been described already, but we have found no paper upon it, and, if one exists, it cannot be generally known, because Soxhlet's extractor is still in common use.

It is made up of apparatus found in every organic laboratory, a 250 cc. flask (or one of larger capacity) fitted with a return condenser, and a piece of wide glass tube open at both ends, which holds the solid to be extracted between two wads of filter paper. A better form is given to the apparatus, if the piece of tube is drawn to a smaller diameter at the bottom, so as to hold a small perforated porcelain plate which, with a single thickness of filter paper, supports the solid in the tube, as in this way a more rapid flow of the extracting liquid is secured. The tube after being loaded with the solid is placed in the flask in a slightly inclined position, and should be long enough to reach from the bottom of the flask nearly to the top of its neck; it stands directly under the return condenser, and receives the liquid dropping from it. There should be sufficient space between the tube and the neck of the flask to allow free passage to the vapor of the solvent. The solid in the tube should stand above the liquid in the flask, and there should be

an empty space 1-3 cm. long above the solid to receive the liquid from the condenser. The success of the process depends



on the adjustment of the filter papers, so as to allow a rapid flow through the tube but with the space above the upper filter kept nearly full of the liquid, as in this way the formation of special channels in the solid is in great measure prevented. This adjustment is a very simple matter, and, after the boiling of the solvent has been started at the proper rate, the apparatus runs automatically.

In order to compare the efficiency of our extractor with that of Soxhlet three series of experiments were tried: the first with 10 grams of anthracene and ether, the extraction being carried on for ten minutes, after which the solvent was evaporated off, and the extract weighed.

Anthracene.

	I.		
	Grams.	Grams.	Grams.
Soxhlet	1.5	2.1	1.9
New extractor	2.8	2.9	

As in these experiments all parts of the solid were soluble in the liquid, the conditions were not those of a true extraction; accordingly in a second series 10 grams of ground turmeric were used with ligroin, which removes the oily constituents; and in a third turmeric and ether, which removes the oils and coloring matters. In these cases the extraction lasted one hour, the time being counted from the instant when the first drop of solvent fell upon the solid.

Turmeric.

	II.		III. Ether. Gram.
	Ligroin boiling at 30°-60°. Gram.	Ligroin boiling at 40°-50°. Gram.	
Soxhlet	0.2	0.2	0.9
New extractor	0.4	0.5	1.3

The agreement of the parallel experiments is as close as could be expected, since it is impossible to secure absolutely similar conditions, and is accurate enough for our purpose, as the largest difference between results with the same extractor is smaller than the smallest difference between the different extractors. These experiments show that our apparatus is more efficient than that of Soxhlet, the amount dissolved by it in some cases being more than twice as great. This is not surprising, as in it the solid is surrounded by the hot vapor of the solvent, which therefore acts at a much higher temperature than in the older form of extractor.

The considerable difference between the amounts extracted from turmeric by ligroin boiling from 30° to 60° and that boiling between 40° and 50° is probably due to the fact that the solid is kept hot by the vapor of the ligroin in the flask, so that much of the lower boiling solvent evaporates away from the tube without passing through it. This difference is much less marked in the Soxhlet extractor, but in spite of this advantage our form is much more effective.

In order to see if the felt thimble used in the Soxhlet apparatus gave as good results as the tube in our form, two extractions were carried on, in which the thimble was substituted

for the tube in our extractor. With ether and anthracene we obtained equally good results from the two forms of holder,

Thimble	2.9	
Tube	2.9	2.8

but in this experiment the conditions were not those of a true extraction, in which the soluble substance is mixed with much inert matter, and when ligroin boiling at 40°-50° and turmeric were used the result was very different.

Thimble	0.3
Tube	0.8

In this case the tube gives a much better result, as indeed was to be expected, because with the felt thimble a large part of the solvent must escape through the sides without coming in thorough contact with the solid, whereas with the tube each drop of the liquid can return to the flask only by passing through the entire column of the solid. In the first case, therefore, much of the solvent will return to the flask without having taken up its full share of the extract, and the lower and inner parts of the solid will be exposed to the action of only a portion of the solvent. The superiority of our apparatus to that of Soxhlet undoubtedly is due to this difference in the holders of the solid as well as to the higher temperature, at which the extraction takes place.

The advantages of our extractor over the Soxhlet form are: (1) The extraction takes less time, in some cases running more than twice as rapidly. (2) A smaller amount of the solvent can be used. Our apparatus runs well with 40-50 cc. of liquid; with Soxhlet's at least 100 cc. are necessary. (3) Our extractor is much the cheaper, being easily put together with pieces of apparatus common in every organic laboratory. (4) It is much less fragile. (5) It is more compact, and therefore more easy to set up firmly than the top-heavy Soxhlet apparatus. (6) It is well adapted to extractions with solvents having high boiling points, which could hardly be used with the Soxhlet form.

CAMBRIDGE, MASS.,
April, 10, 1907.

From the Division of Chemistry, Hygienic Laboratory, U. S. Public Health and
Marine Hospital Service, Washington, D. C.

THE COMBUSTION OF SULPHUR IN AIR AND OXYGEN.

BY J. H. KASTLE AND J. S. MCHARGUE.

Sulphur dioxide has long been employed as a disinfectant and germicide. For many purposes it is regarded as highly efficient and in general its mode of action is sufficiently well understood. Like other substances employed for purposes of disinfection its efficiency is dependent on various conditions such as temperature, moisture, etc., and to some of these its relation is still not well understood. An experimental study of its mode of action as a germicide has therefore been recently undertaken in the Hygienic Laboratory, and the observations herein described were made in connection with this investigation. Ordinarily, the sulphur dioxide required for disinfection is obtained by burning sulphur in air in pots or furnaces, the latter specially designed for this purpose. It therefore became a matter of interest in this connection to determine whether sulphur dioxide is the sole product of the combustion of sulphur, and especially to determine whether any sulphur trioxide is formed during the combustion.

According to Hempel¹ when sulphur is burned in oxygen the quantity of sulphur trioxide produced increases with increase in the pressure of the oxygen to 40 to 50 atmospheres, when approximately half of the sulphur is converted into the trioxide. At still higher pressures the quantity of sulphur trioxide formed is less than this, and at atmospheric pressure the gas resulting from the combustion of sulphur contains 2 per cent of sulphur trioxide and 98 per cent of sulphur dioxide.

According to Hempel, in order to determine the several amounts of these two substances in the gases resulting from the combustion of sulphur, the outlet tube of the autoclave was opened and the sulphur dioxide absorbed by drawing the gases through an absorption flask containing iodine solution. In order to effect the most complete separation of the sulphur dioxide from the trioxide, the autoclave was placed in hot

¹ Ber. d. chem. Ges., **23**, 1455-1457.

water. The sulphur dioxide produced was determined by titration of the iodine solution and the sulphuric acid remaining in the autoclave was determined gravimetrically. According to this author, an exact agreement of the results could not be obtained.

Theoretically, at least, it would seem possible to determine the quantities of sulphur dioxide and trioxide in a mixture of gas by determining (1) the quantity of sulphur dioxide by means of decinormal iodine and (2) the sulphur trioxide by a gravimetric determination of the total sulphur in the solution after absorption. In order, however, that these methods may be employed to advantage in the actual study of sulphur-dioxide disinfection it is essential that they be rapid and easy of execution. The attempt was made therefore to determine both the sulphur dioxide and trioxide volumetrically. This we have been able to accomplish in two ways: (1) by passing the gases resulting from the combustion of the sulphur through a solution of decinormal iodine. In such a solution the sulphur dioxide is absorbed by conversion into sulphuric acid and any sulphur trioxide present in the gas is absorbed by the water which is present. In our earlier work on this subject the iodine solution contained in the several absorption flasks was poured into a measuring flask of convenient volume and made up to the mark with water. In an aliquot part of this solution the excess of iodine was determined by means of decinormal thiosulphate and in another aliquot part of the solution the excess of the iodine was removed by means of a freshly prepared solution of sulphur dioxide of known strength and the total acidity of the resulting solution determined by means of decinormal sodium hydroxide, with phenolphthalein as the indicator. This method depends upon the reaction represented by this chemical equation: $I_2 + SO_2 + 2H_2O = 2HI + H_2SO_4$, and hence for every equivalent of iodine used up theoretically 2 equivalents of acid should result, or, if 10 cc. of a decinormal solution of iodine be used up in this process, 20 cc. of decinormal sodium hydroxide should be required to neutralize the resulting solution exactly. If now a larger quantity than 20 cc. of decinormal sodium hydroxide be re-

quired, the excess is a measure of the sulphur trioxide resulting from the combustion of the sulphur, provided the solution of sulphur dioxide employed in the removal of the excess of iodine does not contain any sulphuric acid. By this method we were able to measure the quantity of sulphur trioxide formed in the burning of sulphur and to prove to our satisfaction that a certain amount of this substance did result in the combustion of sulphur.¹ This method has the disadvantage, however, that the solution of sulphur dioxide has to be prepared fresh every day, and the strength of the solution was found to be very subject to change and hence had to be determined practically for each determination. Fortunately, for the purpose of this investigation it was found that both of these gases could be determined (2) by means of a decinormal iodine solution, decinormal thiosulphate and decinormal sodium hydroxide without resorting to the solution of sulphur dioxide. The method finally employed in our determinations is as follows:

The gases resulting from the combustion of the sulphur are absorbed in a known volume of decinormal iodine solution. The liquid is then poured into a measuring flask of convenient volume and made up to the mark with water. In an aliquot part of this solution the excess of iodine is determined by means of a decinormal thiosulphate, and in this same portion the acid is determined by means of decinormal sodium hydroxide, phenolphthalein being the indicator. The quantity of iodine originally employed minus the quantity remaining unchanged as indicated by the thiosulphate gives the quantity of sulphur dioxide, and the excess of acidity over and above the amount which should result from the action of the iodine on the sulphur dioxide gives the sulphur trioxide resulting from the burning of the sulphur. Obviously, this method is based on well-known reactions employed in iodimetry and acidimetry, and depends finally on the fact that the substances resulting from the action of iodine on sodium thiosulphate are neutral to phenolphthalein. The exact strength of the thiosulphate solution used was determined by means

¹ That small quantities of sulphur trioxide are formed during the combustion of sulphur is evident from the fact that during the combustion, oily drops of sulphuric acid make their appearance on the walls of the tube.

of Kahlbaum's resublimed iodine immediately before each experiment, and the exact strength of the iodine solution determined by means of the thiosulphate. The exact strength of the sodium hydroxide was frequently determined by titrating against weighed amounts of pure oxalic acid. The strict observance of these precautions is necessary if concordant results are to be obtained by this method. A specimen of crystallized sulphur was selected for the investigation. This was twice distilled and each of the first portions of the distillate were rejected. The product thus obtained was broken up into small pieces about the size of bird shot and preserved in a weighing bottle. In all our determinations 0.1 gram of this was weighed out in a porcelain boat, which was then placed in a piece of Jena glass combustion tubing, 30 inches in length. One end of this long tube was drawn down to a tube of smaller caliber and connected with a train of five Drexel absorption bottles, the first four of which contained known amounts of tenth-normal iodine solution, and the last one from the tube contained a 2 per cent solution of potassium iodide. The last absorption bottle was connected with an aspirator bottle. The porcelain boat containing the sulphur was placed about four inches from the farther end of the tube away from the absorption bottles, and this end of the tube was then closed with a cork through which passed a piece of glass tubing about three-sixteenths inch in diameter, one end of which was drawn down so as to give an opening about one-sixteenth of an inch in diameter into the larger tube. Through the smaller tube the air or oxygen required in the combustion of the sulphur was drawn by the aspirator bottle at the end of the train of absorption flasks. Care was taken so to regulate the combustion of the sulphur as to insure complete combustion and no volatilization to the colder parts of the tube. The tube was of such length as to insure complete combustion of the sulphur long before any small amounts that might be volatilized could reach the absorption bottles. In a few of our experiments the sulphur to be burned was spread along the combustion tube over a considerable length, but always from the absorption bottles.

After the combustion was complete, the contents of the tube and bottles were transferred to a measuring flask and made up to the mark with the washings from the tube and absorption bottles. An aliquot portion was then titrated with sodium thio-sulphate. From the number of cc. of iodine used up, the weight of the sulphur burned to sulphur dioxide was calculated. After adding a few drops of phenolphthalein, the acidity of the solution was determined by means of $N/10$ sodium hydroxide. The number of cc. of caustic soda required was recorded as "found acidity." From the quantity of iodine entering into reaction with the sulphur dioxide, the acidity resulting from the conversion of sulphur dioxide into sulphuric acid by means of iodine can be calculated. The found acidity minus the calculated acidity gives the acidity due to sulphur trioxide. From this the weight of the sulphur burned to sulphur trioxide may be calculated, and this, added to the weight of the sulphur burned to sulphur dioxide, gives the total weight of the sulphur burned. The results of our determinations are given in the table on the following pages:

It will be seen from an examination of the results given in the table (1) that there is a reasonable agreement between our determinations and those of Hempel, of the several amounts of sulphur dioxide and sulphur trioxide which result from the burning of sulphur in oxygen, especially when the fact that the combustion was carried out in a somewhat different manner in the two cases is kept in mind. According to Hempel, 2 per cent of the sulphur is burned to sulphur trioxide; according to our determinations, Series IV., 2.76 per cent of sulphur is burned to the trioxide.

(2) It is further evident from our results that considerably larger amounts of sulphur trioxide are formed when sulphur is burned in air than when it is burned in oxygen. That such is the case may be seen from the results given in Series I., II., III., IV., and V. From an average of all of our results bearing on this point, it will be seen that about 2.5 times as much sulphur is burned to sulphur trioxide in air as is burned to sulphur trioxide in oxygen.

(3) The results obtained in Series I., II., III., and in the

Results of Experiments on the Combustion of 0.1 Gram of Sulphur in Air and Oxygen under the Conditions Indicated in the Table.

Number of experiment.	Conditions.	Found.		Total.	Per cent of sulphur burned to SO ₂ .	Per cent of sulphur burned to SO ₂ .	Total per cent of sulphur found.	
		Sulphur burned to SO ₂ .	Sulphur burned to SO ₂ .					
1	In air	0.09426	0.006884	0.101144	94.26	6.88	101.14	
2	"	0.09288	0.007614	0.100494	92.88	7.61	100.49	
3	"	0.09183	0.007690	0.09952	91.83	7.69	99.52	
Average, Series I.				0.09299	0.007396	0.100386	92.99	100.38
4	In moist air	0.09153	0.00787	0.0994	91.53	7.87	99.40	
5	"	0.09578	0.00413	0.0999 ¹	95.78	4.13	99.91	
6	"	0.09153	0.00787	0.0994	91.53	7.87	99.40	
Average, Series II.				0.09294	0.00662	0.09956	92.94	99.57
7	In air dried over sulphuric acid and lime	0.09049	0.009510	0.1000	90.49	9.51	100.00	
8	In air dried over sulphuric acid and lime	0.09373	0.006700	0.10043	93.73	6.70	100.43	
9	In air dried over sulphuric acid and lime	0.09316	0.006020	0.09918	93.16	6.02	99.18	
10	In air dried over sulphuric acid and lime	0.09456	0.005214	0.099774	94.56	5.21	99.77	
Average, Series III.				0.09298	0.006888	0.099868	92.98	99.87

¹ By difference.

Results of Experiments on the Combustion of 0.1 Gram of Sulphur in Air and Oxygen under the Conditions Indicated in the Table (Continued).

Number of experiment.	Conditions.	Found.		Per cent of sulphur burned to SO ₂ .	Per cent of sulphur burned to SO ₂ .	Total per cent of sulphur found.
		Sulphur burned to SO ₂ .	Sulphur burned to SO ₂ .			
11	In dry air containing large quantity of carbon dioxide					
12	In dry oxygen	0.09309	0.00657	0.09966	6.57	99.66
13	"	0.09600	0.00314	0.09914	3.14	99.14
14	"	0.09704	0.00240	0.09944	2.40	99.44
15	"	0.09790	0.00270	0.1006	2.70	100.60
16	"	0.09608	0.00347	0.09955	3.47	99.55
17	"	0.09665	0.00257	0.09922	2.57	99.22
	"	0.09698	0.00230	0.09928	2.30	99.28
	Average, Series IV.	0.09677	0.00276	0.09953	2.76	99.53
18	In moist oxygen	0.09893	0.00250	0.10143	2.50	101.43
19	"	0.09776	0.00269	0.10045	2.69	100.45
20	"	0.09720	0.00278	0.09998	2.78	99.98
	Average, Series V.	0.09796	0.00266	0.10062	2.66	100.62

Results of Experiments on the Combustion of 0.1 Gram of Sulphur in Air and Oxygen under the Conditions Indicated in the Table (Continued).

Number of experiment.	Conditions.	Found.		Per cent of sulphur burned to SO_2 .	Per cent of sulphur burned to SO_3 .	Total per cent of sulphur burned found.
		Sulphur burned to SO_2 .	Sulphur burned to SO_3 .			
21	In a mixture of oxygen and carbon dioxide	0.09488		94.88		
22	In a mixture of oxygen and carbon dioxide	0.09763		97.63		
23	In a mixture of oxygen and carbon dioxide	0.09577		95.57		
24	In a mixture of oxygen and carbon dioxide	0.09689		96.89		
25	In a mixture of oxygen and carbon dioxide	0.09778		97.78		
26	In a mixture of oxygen and carbon dioxide	0.09743		97.43		
Average, Series VI.		0.09673	0.00327 ¹	96.73	3.27 ¹	100.00 ¹

¹ By difference.

Results of Experiments on the Combustion of 0.1 Gram of Sulphur in Air and Oxygen under the Conditions Indicated in the Table (Continued).

Number of experiment.	Conditions.	Found.		Per cent of sulphur burned to SO ₂ .	Per cent of sulphur burned to SO ₂ .	Per cent of sulphur burned to SO ₂ .	Total per cent of sulphur found.
		Sulphur burned to SO ₂ .	Sulphur burned to SO ₂ .				
27	In equal parts by volume of oxygen and nitrogen	0.09552	0.0057	0.10122	95.52	5.70	101.22
28	In equal parts by volume of oxygen and nitrogen	0.09233	0.0077	0.10003	92.33	7.70	100.03
	Average, Series VII.	0.09392	0.0067	0.10062	93.92	6.70	100.62
29	In a mixture of 3 parts of oxygen and 1 part of nitrogen	0.09694	0.0029	0.09984	96.94	2.90	99.84
30	In a mixture of 3 parts of oxygen and 1 part of nitrogen	0.09901	0.0023	0.10131	99.01	2.30	101.31
	Average, Series VIII.	0.09797	0.0026	0.10057	97.97	2.60	100.57

single experiment No. 11 would seem to indicate that moisture, dryness, and the presence of increased amounts of carbon dioxide are practically without influence on the combustion of sulphur in air and from Series IV. and V. it would seem that moisture is practically without influence on the combustion of sulphur in oxygen and from Series VI. that carbon dioxide exerts but little influence on the combustion of sulphur in oxygen, the amount of sulphur trioxide being only slightly increased over the amount of this substance formed in dry and moist oxygen.

Finally, it would seem from the results of our experiments given in Series VII. that the increased production of sulphur trioxide in air as compared with oxygen is due in some way to the presence of nitrogen. It will be seen further from Series VIII. that a great diminution in the quantity of nitrogen as compared with the quantity present in the experiments of Series VII. and the quantity normally present in air causes a marked falling off in the production of sulphur trioxide.

It would seem therefore from our results that in the combustion of sulphur, nitrogen acts as a carrier of oxygen.

In support of this conclusion the fact may be mentioned that in the combustion of certain substances in air small amounts of nitrogen are also oxidized. For example, Kämmerer¹ proved the presence of nitrogen peroxide in the gases left after burning magnesium in air, by the characteristic odor and color of the gas and by the formation of the iodide of starch on the addition of the potassium-iodide-starch reagent. Dixon and Russell² have also observed that when an explosive mixture of carbon bisulphide and oxygen, containing small amounts of nitrogen, is ignited, some of the nitrogen is invariably oxidized.

The formation of small amounts of one of the higher oxides of nitrogen during the burning of sulphur in air and the subsequent oxidation of sulphur dioxide thereby would afford the simplest explanation of the fact that considerably more sulphur trioxide is formed when sulphur is burned in air and in

¹ Ber. d. chem. Ges., **10**, 1684.

² Jour. Lond. Chem. Soc., Trans., 1899, pp. 75-610.

mixtures of oxygen and nitrogen than is produced when it is burned in pure oxygen or in mixtures of carbon dioxide and oxygen.

ON SOME CUPRAMMONIUM SALTS, IV.

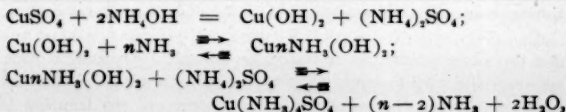
CUPRAMMONIUM SULPHATE.

BY DAVID W. HORN.

The object of this paper is to describe a series of experiments of interest in connection with a recent paper by Dawson on "The Nature of Ammoniacal Copper Solutions."¹

Dawson describes a continuation, under more favorable conditions, of the work of Dawson and McCrea,² in which the ratios between "free" and "combined" ammonia were determined in solutions of copper sulphate to which an excess of ammonia had been added. In determining the free ammonia they took advantage of a method previously used by Hantzsch and Sebaldt³ and found the amounts extracted when the solutions were shaken with chloroform.

At the close of this recent paper, the author says "although the exact nature of the changes is not yet known, yet the experimental facts are in agreement with the equations.



n being less than 4 and probably equal to 2. It is hoped that experiments in progress will throw further light on the nature of the complex system." The experiments to be described in this paper were carried out to determine the exact nature of one of the changes referred to.

After careful study of the compound $\text{CuSO}_4 \cdot 4\text{NH}_3 \cdot \text{H}_2\text{O}$ it was apparent that the behavior of a solution of this compound at

¹ J. Chem. Soc., 89, 1666 (1906).

² *Ibid.*, 77, 1239 (1900); 79, 493, 1069, 1072 (1901).

³ Z. physik. Chem., 30, 258 (1899).

0.1 N concentration was different from that of a solution of the same concentration prepared by adding the necessary amount of ammonia to a 0.1 N solution of copper sulphate.¹ This difference in behavior was particularly interesting because in all the physical-chemical studies bearing on the nature of ammoniacal copper sulphate solutions made up to that time, the solutions had been prepared from copper sulphate solutions by adding ammonia.² For this reason the experiments described in the previous paper were carried somewhat further. The results acquire a fresh interest since Dawson, in his most recent work, has examined two 0.1 N solutions of the compound $\text{CuSO}_4 \cdot 4\text{NH}_3 \cdot \text{H}_2\text{O}$, and has found that "the data agree satisfactorily with those obtained in the examination of solutions containing larger quantities of ammonia," i. e., solutions prepared by adding an excess of ammonia to solutions of copper sulphate. Of these latter solutions the one that contained least ammonia had in it 5.48 mols. NH_3 to 1 atom Cu.

The Change in a 0.1 N Cuprammonium Sulphate Solution.

If a solution of copper sulphate is prepared and ammonia and water added to it until the concentration with respect to copper is 0.1 N and to ammonia 0.4 N, the solution may be kept in a stoppered bottle at least a year without any apparent change taking place in the solution. If a solution of the compound $\text{CuSO}_4 \cdot 4\text{NH}_3 \cdot \text{H}_2\text{O}$ that is 0.1 N with respect to copper, is kept under the same conditions it begins to deposit a crystalline blue substance in visible quantity after about 32 hours. This deposition continues for some time, but if some of the solution is filtered after a couple of months it furnishes no more deposit though kept a year afterward, even when in contact with a small amount of the crystallized blue substance.

The change that occurs in solutions of the compound $\text{CuSO}_4 \cdot 4\text{NH}_3 \cdot \text{H}_2\text{O}$ is not due to any inherent instability in this compound itself. The compound may be kept in a desiccator over

¹ Horn and Taylor: *THIS JOURNAL*, **32**, 279H.

² Reychler: *Ber. d. chem. Ges.*, **28**, 555 (1895). Konowaloff: *J. Russ. Phys. Chem. Soc.*, **31**, 910 (1899); *Centralbl.*, 1900, **1**, 938. Gans: *Z. anorg. Chem.*, **25**, 236 (1900). Dawson and McCrea: *Loc. cit.* (1900, 1901). Bonzat: *Ann. Chim. Phys.* [7], **29**, 305 (1903). Locke and Forssall: *THIS JOURNAL*, **31**, 268 (1904). *vide also* Blanchard: *J. Am. Chem. Soc.*, **26**, 1315 (1904). Bonsdorff: *Z. anorg. Chem.*, **41**, 132 (1904).

lime at least a year. The percentages found upon analysis of a specimen thus kept were:

Cu	25.85	25.87	25.88
NH ₃	27.74	27.82	27.77

The percentages required by the formula are 25.86 and 27.75 respectively.¹ It may be kept in a desiccator over phosphorus pentoxide at least a week; 0.80753 gram was placed in a weighing tube previously dried to constant weight over the pentoxide. The total weight was 12.28788 grams. After the open tube containing the compound had been in a desiccator with pentoxide a week, the total weight was 12.28793 grams. Therefore the tensions of ammonia and water over the compound are practically *nil*, and for a hydrated salt and an ammonia compound of copper this cuprammonium sulphate is exceptionally stable.

When the solid is exposed to water, even the water-vapor in the air, it begins to evolve ammonia at once. In the air, white fumes of ammonium chloride are formed as soon as a rod moistened with a solution of hydrochloric acid is brought over the salt, and the odor of ammonia is appreciable within a minute or two.

In a solution of the salt a change must, then, begin immediately. It was shown in the previous paper that a change goes on for some time in the solution. This change with time has since been studied more closely.

A 0.1 N solution of the compound was prepared by weighing the compound in a calibrated measuring-flask, and later adding water gradually while the flask was shaken. The exact strength of the solution was 0.0998 N. Several portions of the solution were sealed up in glass tubes and kept at constant temperature in a bath. A tube was removed at regular intervals and its contents examined. The examination consisted in titrating the solution into a standard acid, taking as end reactions the formation of a permanent precipitate and the acidity as indicated by methyl orange. These end reactions have been discussed in a previous paper.² Since then it has been shown that these

¹ Horn and Taylor: *Loc cit.*, p. 267.

² *Loc. cit.*, p. 281ff.

end reactions are characteristic of cuprammonium sulphate throughout a wide range of concentration. It will be seen from columns V. and VI. in the following table that the end reactions are independent of the concentration between 0.5 N and 0.05 N.

Table I.

Number of experiment.	N in fraction X expressing normality to Cu.	Cc. acid required by cc. solution to produce end reaction by precipitation.	Cc. acid required by 1 cc. solution to produce end reaction by methyl orange.	By precipitation, molecules of ammonia neutralized for every 1 gram atom of Cu added to acid.	By methyl orange, molecules of ammonia neutralized for every 1 gram atom of Cu added to acid.
I.	II.	III.	IV.	V.	VI.
1	2.006	9.023	9.348	3.860	3.999
2	1.998	9.024	9.340	3.845	3.980
3	5.015	3.615	3.710	3.865	3.967
4	4.996	3.621	3.720	3.858	3.963
5	7.518	2.398	2.479	3.844	3.974
6	7.514	2.401	2.488	3.847	3.986
7	10.032	1.809	1.855	3.869	3.968
8	10.026	1.810	1.856	3.869	3.968
9	13.333	1.357	1.392	3.857	3.956
10	14.953	1.212	1.247	3.865	3.976
11	15.236	1.195	1.221	3.882	3.967
12	20.003	0.912	0.933	3.894	3.984
13	19.990	0.912	0.935	3.878	3.976

Using these end reactions, the changes observed in the 0.1 N solution of the compound $\text{CuSO}_4 \cdot 4\text{NH}_3 \cdot \text{H}_2\text{O}$, kept at $23^\circ.6 \pm 0^\circ.1$, were as follows:

Table II.

No.	Time. Hours.	Cc. precipitation.	Cc. methyl orange.	X NH_3 : 1 Cu precipitation.	X NH_3 : 1 Cu methyl orange.
I.	II.	III.	IV.	V.	VI.
1	0	1.915	1.971	3.865	3.978
2	8.5	1.909	1.950	3.852	3.938
3	20.5	1.893	1.948	3.820	3.931
4*	32.5*	1.896*	1.952*	3.826*	3.939*
5	44.5	1.880	1.920	3.794	3.875
6	56.5	1.854	1.922	3.741	3.878
7	68.5	1.831	1.869	3.695	3.772
8	80.5	1.795	1.847	3.622	3.727

The figures in the several columns have the same meaning as in Table I., except those in column II., which refer to the time between the preparation of the solution and its analysis. The asterisks indicate the time when a deposit of the blue crystallized substance was first noticed.

The results in Table II. show that a gradual change continues for some time in the solution, and that during that time the crystallized blue product appears.

Composition of the "Crystallized Blue Substance."

To obtain a fair amount of this product, 5 liters of 0.1 N solution were prepared and allowed to stand in the dark for one year. The product, when separated, was pressed gently between filter papers to remove adhering mother liquor, and dried for a week over lime. It was analyzed by methods that have been described in detail.¹ The results follow (p. 480).

These analyses require some discussion. There is, of course, no difficulty in determining the copper by Rose's method, but the quantitative separation of sulphuric acid from copper is extremely difficult, although the same constituents are separated readily by the same method when the compound analyzed is $\text{CuSO}_4 \cdot 4\text{NH}_3 \cdot \text{H}_2\text{O}$. Every attempt made to accomplish this separation when the blue basic salt was in *acid* solution before it was added to the potassium hydroxide failed even when the basic salt was dissolved in concentrated hydrochloric acid and digested with it hot for several hours and in the cold for 24 hours. Although dissolved, the sulphuric acid and copper do not seem to separate, at least entirely, from their former union in the dry salt. If the basic salt is dissolved in concentrated ammonia water, the difficulty in separation disappears. This is not due simply to the alkalinity of the solvent, for when an acid solution of the basic salt is mixed and digested for several hours with a boiling solution of potassium hydroxide, the copper hydroxide precipitate persistently retains sulphuric acid. Analyses 8 and 9 for ammonia are to be given preference to 6 and 7, for in 6 and 7 only about 5 cc. of standard acid were neutralized in the distillations with sodium hydroxide, and this is a small quantity

¹ Horn and Taylor: *Loc. cit.*, p. 254-61.

Table III.

No. of substance anal. taken for ysis. analysis.	Weight of CuS found.	Cc. acid neutralized.	Weight BaSO ₄ found.	Loss in weight found.	CuO found. Per cent.	NH ₃ found. Per cent.	SO ₃ found. Per cent.	H ₂ O found. $\frac{2}{3}$ NH ₃ . $\frac{1}{2}$ H ₂ O. Per cent.	Percentages calculated for 4CuO. 8O ₃ . found. $\frac{2}{3}$ NH ₃ . $\frac{1}{2}$ H ₂ O. Per cent.
1	0.2539				61.00				61.00
2	0.1996				60.99				
3	0.2539		0.1173				15.84		15.34
4	0.2824		0.1301				15.80		
5	0.2985		0.1373				15.78		
6	0.1936	4.78				8.99			8.69
7	0.2025	5.05				9.07			
8	1.0060	24.03				8.69			
9	1.1128	26.57				8.69			
10	0.3754			0.0877	23.37 23.37 — 8.69 =			14.68	14.96
					Loss in weight. Per cent.				

over which to distribute the necessary error in the volumetric work. Analysis 10 involved an attempt to drive off the ammonia and water and determine them separately in the mixed vapor. The basic salt had to be heated with lead oxide to retain its sulphuric acid. Either this lead oxide, or the copper oxide of the salt, or the oxygen of the air passed through the heated tube oxidized part of the ammonia, or else part of the ammonia was broken down and left the compound as nitrogen when the compound was heated. Only 5.22 per cent out of a total 8.69 per cent appeared as ammonia and was absorbed by the dilute sulphuric acid in the absorption apparatus, *i. e.*, only about 0.6 of the total. A reducing action of the escaping ammonia when similar basic cuprammonium salts are heated has already been suggested by Pickering.¹ However, the total loss in weight of the boat was 23.37 per cent of the weight of the original salt. This must represent very nearly the sum of the weight of water and ammonia originally in the salt, for the loss in weight that would occur if the ammonia oxidized were all oxidized at the expense of the copper oxide is negligible, inasmuch as the total ammonia oxidized is only 0.035 of the total weight of the salt heated. Subtracting from this loss in weight the percentage of ammonia found by distillation, the percentage of water is found to be 14.68. If the sum of the percentages of CuO, SO₃ and NH₃ are subtracted from 100, the percentage of water is found to be 14.50. It is fair to assume, then, that the basic salt contains about 14.7 per cent water. As compared with the calculated values column V., Table III., this percentage is low. This is to be expected since the percentage of SO₃ is high. When the difficulties in the analyses are considered, the variation is not great in either case.

The analytical results lead to a formula so complex that it was adopted most reluctantly. It is retained as the only formula that satisfies the requirements of other experimental data to be given later, as well as the analytical results in Table III. This formula is $4\text{CuO} \cdot \text{SO}_3 \cdot 2\frac{2}{3}\text{NH}_3 \cdot 4\frac{1}{3}\text{H}_2\text{O}$.

The Residual Copper in the Solution.

When the original 0.1 N solution had been kept about 2 months

¹ J. Chem. Soc., 43, 399 (1883).

and had deposited the blue basic salt, some of the clear supernatant liquid was siphoned off and divided into two parts. One part was kept by itself in a bottle for over a year. The other part was kept the same length of time in another bottle in contact with a small quantity of the crystallized basic salt. In neither was more basic salt deposited. Therefore, the change by which the basic salt was produced had reached a definite limit with time.

In order to find out how much basic salt had been formed, the solution from which it had been deposited was analyzed for copper. The results follow:

Weight of solution taken for analysis. Gram.	Weight of Cu_2S found. Gram.	Weight of Cu in 1 gram of solution. Gram.	Volume of solution taken for analysis. cc.	Sp. gr. of solution referred to water at 4° .
21.1588	0.11199		21.075	1.0055
21.1545	0.11080		21.075	1.0057
Average, 21.1566	0.11139	0.004205	21.075	1.0056

The solution was measured from a carefully calibrated 25 cc. burette, from 0 to the 21 cc. mark. The delivering capacity of the burette to this mark was 21.075 cc. of water at 20° . The specific gravity of the solution was calculated, using Rossetti's value, 0.998259, for the density of water at 20° referred to water at 4° . One liter of the filtered solution weighed 1005.6 grams and contained 4.2288 grams of copper. One liter of the original 0.1 N solution contained 6.36 grams of copper. Therefore, 0.665 or $\frac{2}{3}$ represents the fraction of the original copper that has remained in the solution, and $\frac{1}{3}$ the fraction deposited as basic salt.

It was desirable to determine in what form the $\frac{2}{3}$ of the original copper existed in the filtered solution. It is, of course, impossible to do this if the problem is construed in its strictest sense. But it was found that very nearly all of the residual copper is separated as a crystalline compound upon the addition of alcohol. As judged by its color the copper not thus separated is very small and may fairly be assumed to be due to the quantity of crystallized compound required to saturate the alcoholic mother liquor.

This compound appears in fine needles that have a blue color approaching to that of the lilac. Its appearance is not suggestive of any cuprammonium compound known in this laboratory. The following were found to be the best conditions for its preparation:

Two hundred cc. of the hydrolyzed solution were placed in a narrow bottle and an efficient form of glass stirring-rod was rotated rapidly about the axis of the bottle by means of a Rabe turbine; 250 cc. of 95 percent alcohol were added from a pipette, requiring about 3 minutes for addition. The stirring was continued about half a minute after the alcohol had been added. The liquid, which was slightly turbid, was set aside, and soon began to deposit brilliant, fine needles. After 16 hours these crystals were collected, gently pressed between filter papers until almost dry, and finally put between fresh papers in a screw press. As soon as dry, they were weighed for analysis. This substance and the basic salt form a compact cake if pressed hard when wet, and when this has formed the compound cannot be dried before it has decomposed. The following are analyses of two different specimens (see p. 484).

In spite of its unusual appearance, this crystallized lilac compound had the composition of ordinary cuprammonium sulphate, $\text{CuSO}_4 \cdot 4\text{NH}_3 \cdot \text{H}_2\text{O}$. It contained a little basic salt—perhaps the cause of the turbidity that existed before the crystals were formed.

The Residual Sulphuric Acid in the Solution.

Since $\frac{1}{3}$ of the original cuprammonium sulphate had been converted into the compound $4\text{CuO} \cdot \text{SO}_3 \cdot 2\frac{2}{3}\text{NH}_3 \cdot 4\frac{1}{3}\text{H}_2\text{O}$ and $\frac{2}{3}$ was recovered in the original form, $\frac{1}{4}$ of the total SO_3 must have remained in the solution in some other form. The solution always had the odor of ammonia and, therefore, always contained free ammonia, so that it is likely that this $\frac{1}{4}$ of the SO_3 formed ammonia sulphate as rapidly as it was set free.

The Chemical Equation.

The equation that represents all of the facts thus far presented that bear upon the quantities involved in the change is

Table IV.

	Weight of substance taken for analysis. Gram.	Weight Cu_2S found. Gram.	Cc. acid neutralized.	Weight BaSO_4 found. Gram.	Cu found. Per cent.	NH_3 found. Per cent.	SO_4 found. Per cent.	Percentages calculated for $\text{CuSO}_4 \cdot 4\text{H}_2\text{O}$.
A	0.2975	0.0983			26.40			25.86
	0.2513	0.0832	18.60		26.43	27.34		27.75
	0.2491		16.99			27.36		
	0.2274							
B	0.1926	0.0627			26.00			25.86
	0.2051	0.0669			26.05			
	0.2924	0.0954	20.42		26.05	26.92		27.75
	0.2752		18.03			27.08		
	0.2417						39.08	39.06
	0.2519			0.2393			39.08	
	0.2449			0.2326				

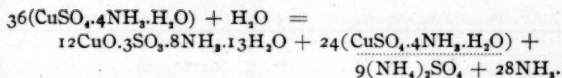
Molecular ratios found by analyses:

Cu : NH_3 : SO_4

A 1 : 3.861

B 1 : 3.867 : 0.994

Calculated, 1 : 4 : 1



The dotted line indicates that the substance thus marked is obtained upon the addition of alcohol.

As will be readily seen from the equation, ammonia exists in three different forms in the solution after the basic salt has separated. To designate these I shall use the terms *free*, *nuclear* and *combined*. Locke has referred to the ammonia combined with copper as "burned," but this does not suggest, as does the word *nuclear*, that the ammonia referred to exists in a complex. Dawson refers to the same as "combined," but this serves better to designate the condition in ammonium sulphate or other ammonium salt.

The equation could be tested by determining the *free* ammonia in the solution after the change was complete but there is, at present, no exact method for making this determination when *nuclear* ammonia is also present. Consequently, the equation was tested by determining the *combined* ammonia.

As the average of 2 analyses 21.121 grams of the solution yielded 0.0061884 gram corresponding to 6.22 grams per litre—the sum of the *free*, *nuclear* and *combined* ammonia.

As the average of 3 titrations, 1 litre of the solution contained 3.333 grams—the apparent sum of the *free* and *nuclear* ammonia. This result is low, of course, since methyl orange indicates but 3.97 out of every 4 molecules of *nuclear* ammonia (see Table I.). Although slightly low it must lie near the true value.

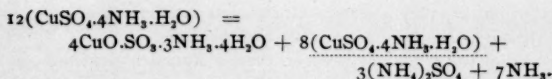
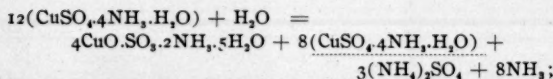
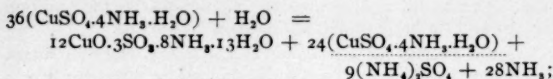
From the difference between these 2 sums the *combined* ammonia is 0.89 gram per liter, and the corresponding value calculated from the equation is 0.85 gram per liter.

If it be assumed that for every copper atom in the solution there are 4 molecules of *nuclear* ammonia, and on this assumption the value found by titration is corrected, the experimental value for the *combined* ammonia becomes 0.85 gram per liter. The only ground for this assumption is the fact that practically all the copper in the hydrolyzed solution separates as the compound, $\text{CuSO}_4 \cdot 4\text{NH}_3 \cdot \text{H}_2\text{O}$, upon the addition of alcohol,

and in the closer agreement between experiment and the equation. The assumption is unnecessary, but the value 0.85 gram calculated upon it is useful in showing that if the methyl orange indicated all the *nuclear* ammonia, instead of 3.97 out of every 4 molecules, the experimental value for the *combined* ammonia would agree very closely with that calculated from the equation.

The analytical values, whose difference gives the combined ammonia, are both low. But they are equally low, which indicates the loss of *free* ammonia by the solution before analysis. As pointed out, the value for the *combined* ammonia is independent of this loss of *free* ammonia. The loss is probably accounted for by the laboratory records which show that the 0.1 N solution was made December 4, 1903; the basic salt was analyzed February, 1905; the lilac salt, May, 1905, and January, 1906; and the solution at the end of January, 1906.

There are, in all, 3 equations that approximately satisfy the requirements of the experiments, *viz.*,

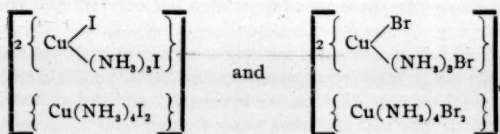


The only ways to determine which of these is the correct one are by determination of the *free* ammonia, and by the analysis of the basic salt. In the absence of an exact method for determining *free* ammonia in such cases, the composition of the basic salt must be the criterion. A comparison of the results of the analysis of the basic salt with the requirements of the several formulae follows:

	Pound. Per cent.	Calculated for $4\text{CuO} \cdot \text{SO}_3 \cdot 2\frac{2}{3}\text{NH}_3 \cdot 4\frac{1}{3}\text{H}_2\text{O}$. Per cent.	Calculated for $4\text{CuO} \cdot \text{SO}_3 \cdot 2\text{NH}_3 \cdot 5\text{H}_2\text{O}$. Per cent.	Calculated for $4\text{CuO} \cdot \text{SO}_3 \cdot 3\text{NH}_3 \cdot 4\text{H}_2\text{O}$. Per cent.
CuO	61.00	61.00	60.92	61.03
SO ₃	15.81	15.34	15.32	15.35
NH ₃	8.69	8.69	6.53	9.81
H ₂ O	14.68	14.96	17.23	13.81

The substance $4\text{CuO} \cdot \text{SO}_3 \cdot 2\text{NH}_3 \cdot 5\text{H}_2\text{O}$ has already been described by Pickering.¹

The formula $4\text{CuO} \cdot \text{SO}_3 \cdot 2\frac{2}{3}\text{NH}_3 \cdot 4\frac{1}{3}\text{H}_2\text{O}$ is the only one that is in accord with all the data obtainable. This formula is like some that have had to be adopted in other cases in order to account for the experimental data. Evidence is steadily accumulating, proving that metal-ammonia compounds of considerable complexity are not uncommon. They occur frequently among cobalt² and chromium compounds.³ Among copper-ammonia compounds, T. W. Richards and Shaw have found⁴ that the substance described by Rammelsberg⁵ as $\text{CuBr}_2 \cdot 3\text{NH}_3$ must be represented as $\text{CuBr}_2 \cdot 3\frac{1}{3}\text{NH}_3$ and Kohlschütter⁶ has analyzed the analogous iodide, $\text{CuI}_2 \cdot 3\frac{1}{3}\text{NH}_3$. Kohlschütter represents these compounds as



following the general methods of representation used by Jörgensen and Werner. In an analogous way, this basic salt may be represented as

¹ *Loc. cit.*

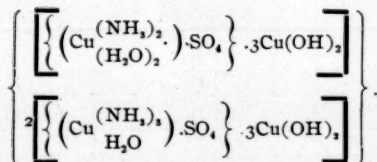
² Jörgensen: *Z. anorg. Chem.*, **5**, 147 (1894).

³ Pfeiffer: *Ann. Chem. (Liebig)*, **343**, 283 (1905).

⁴ *This Journal*, **15**, 651 (1893).

⁵ *Pogg. Ann.*, **55**, 247 (1842).

⁶ *Ber. d. chem. Ges.* **37**, 1157 (1904).



In addition to the experimental facts set forth in the equation, there are one or two others that should be given as bearing upon the changes in ammoniacal copper solutions. When a solution, prepared by adding water and ammonia to dissolved copper sulphate until it was 0.1 N with respect to copper and 0.4 N with respect to ammonia, had been kept a year, the addition of some finely ground basic salt, $4\text{CuO} \cdot \text{SO}_3 \cdot 2\frac{2}{3}\text{NH}_3 \cdot 4\frac{1}{3}\text{H}_2\text{O}$, produced no apparent effect. When a solution prepared in the same way was treated with a little of the basic salt immediately after the solution had been made, it deposited a very considerable quantity of the basic salt within 24 hours. It is possible that the solutions made from cuprammonium sulphate deposit this same basic salt as a result of the formation of a minute quantity of it when the water was added in making the solutions. The turbidity that is produced while these solutions are being made apparently disappears upon thorough mixing, but a quantity of solid sufficient to "seed" the solution might escape observation. Dawson speaks of a small amount of the copper as present in the solutions freshly made from cuprammonium sulphate in the form of "colloidal or suspended copper hydroxide" and it may be that this is the solid basic salt that starts the deposition.

The basic salt $4\text{CuO} \cdot \text{SO}_3 \cdot 2\frac{2}{3}\text{NH}_3 \cdot 4\frac{1}{3}\text{H}_2\text{O}$ is soluble in sufficiently concentrated ammonia water, so that it would not deposit from solutions containing a considerable excess of ammonia. It is deposited, however, when the excess of ammonia is small. I have obtained it from solutions in which the ratios of copper to ammonia were 1 : 4.075 and 1 : 4.1, respectively, but I have not carried the experiments further.

The data in this paper leave no doubt that, under ordinary conditions, the behavior of a freshly prepared 0.1 N solution

of cuprammonium sulphate is different from the behavior of a solution of the same strength prepared from copper sulphate and ammonia.

The lilac and the purple cuprammonium sulphates may not be identical. Cases better adapted to determining whether or not isomerism occurs in such compounds are at present under study.

CHEMICAL LABORATORY,
BRYN MAWR COLLEGE,
January, 1907.

ON THE HYDROLYSIS OF AMIDES BY ACIDS.

FIFTH COMMUNICATION ON CATALYSIS.

BY S. F. ACREE AND SIDNEY NIRDLINGER.

[We are indebted to the Carnegie Institution of Washington for aid in this work.]

From the work of Ostwald¹ and of Remsen and Reid² it has been known that while amides are changed extremely slowly by water they are saponified comparatively rapidly in aqueous solutions of acids, and that the velocity of the reaction is proportional to the concentration of the amide and of the hydrogen ions. Furthermore, the amides are weak bases and form salts. In dilute aqueous solutions, however, these salts are so nearly completely hydrolyzed that only a small amount of salt exists in equilibrium with the amides and the acid. It has been suspected for some time by Kastle,³ Stieglitz⁴ and by Acree⁵ that in the saponification of amides by acids we are dealing with catalytic reactions entirely analogous to the inversion of cane-sugar, formation and saponification of esters, hydrolysis of oximes, etc. It is thought that all these catalytic reactions involve the primary union of the acid with the weak base (amide, ester, organic acid, cane-sugar) and the subsequent saponification of this intermediate salt by water (alcohol).

¹ J. prakt. Chem., **135**, 1.

² THIS JOURNAL, **21**, 281. Reid: *Ibid.*, **24**, 397.

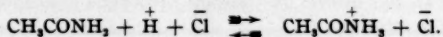
³ THIS JOURNAL, **19**, 894. P. Am. Assn. Adv. Sci., **47**, 238.

⁴ Congress of Arts and Sciences, St. Louis, 1904, Vol. 4, p. 276.

⁵ Acree and Johnson: THIS JOURNAL, **37**, 410; **38**, 258.

This idea of catalysis through intermediate salt formation explains the direct proportionality between reaction velocity and concentration of hydrogen ions on the one hand and the concentration of the intermediate salt and the concentration of hydrogen ions on the other hand, as has been thoroughly discussed in the preceding article by Acree and Johnson.¹ With the idea of applying this hypothesis quantitatively to the saponification of amides, we have taken up the study of the hydrolysis of acetamide by hydrochloric acid.

In a solution containing A gram molecules of acetamide per liter and B gram molecules of hydrochloric acid per liter the amount of salt formed can be readily calculated from the equation worked out by Arrhenius,² Walker,³ and Shields,⁴ and can be represented by the equation:



$$(A-y)(B-y) = K_{hyd} y = \frac{K_w}{K_b} y \quad (1)$$

in which y is the concentration of the small amount of dissociated salt formed, $(A-y)$ the concentration of the acetamide and $(B-y)$ that of the hydrogen ions, when equilibrium is established, K_b the affinity constant of the base and K_w the ion product of water at the temperature. That the acetamide actually does unite with the hydrogen ions and form the salt is shown by the data in Table XVII. The conductivity of 0.1 normal hydrochloric acid was continually lowered by the addition of successive amounts of acetamide. The concentration of the amount of the undissociated salt is so small in comparison with y , since the solution of the salt is very dilute, that it may be neglected at present. During the course of the reaction, when the concentrations of the acetamide and of the hydrogen ions have been decreased by the amount x , equation (1) assumes the form

¹ THIS JOURNAL, 33, 258.

² Z. physik. Chem., 5, 1; 13, 407.

³ Ibid., 4, 319; 33, 137. J. Chem. Soc., 77, 5.

⁴ Z. physik. Chem., 13, 167.

$$(A-x-y)(B-x-y) = K_{hyd} y. \quad (2)$$

When the base is very weak, as is the case with esters, cane-sugar and many amides, y is so small in comparison with $(A-x)$ and $(B-x)$ that equation (2) can be written in form of equation (3) without much error, and the value of y can be approximately calculated very easily. It can always be rigidly calculated,

$$(A-x)(B-x) = K_{hyd} y. \quad (3)$$

however, provided the conditions of equation (1) are known. If now, the salt y is the substance hydrolyzed the velocity of transformation of the acetamide must always be strictly proportional to y according to equation (4)

$$\frac{-dy}{dt} = K_{trans} y \times (C_{H_2O} - x) \quad (4)$$

in which K_{trans} is the reaction velocity and $(C_{H_2O} - x)$ the concentration of the water at the moment considered. But $-dy$, the small amount of salt transformed in dt , is exactly equivalent to dx , the small amount each of acetamide, hydrogen ions, and of water used in forming the end products produced in dt . Furthermore, reaction (1) or (3) is the neutralization of a base by an acid, and this takes place practically instantaneously in comparison with the saponification reaction (4). The value of y , however, can not be determined unless the affinity constant of the acetamide is known. This has been studied by Walker,¹ but the use of Walker's value did not give satisfactory results, and we shall re-determine this value. We can, however, substitute a value involving A , B , and x which is always approximately equivalent to y , and hence avoid the use of y , the real value of which is not known. We may, therefore, substitute at any moment for $-dy$ and y in (4) the corresponding values in equation (3), involving x , and we then obtain

$$\frac{dx}{dt} = \frac{K_{trans} K_b}{K_w} (A-x)(B-x)(C_{H_2O} - x). \quad (5)$$

¹ Z. physik. Chem., 4, 319.

If the solutions are dilute ($C_{H_2O} - x$) is practically constant and then we obtain equation (6) instead of equation (5):

$$\frac{dx}{dt} = K(A-x)(B-x) \text{ or } \frac{1}{t(A-B)} \log \frac{B(A-x)}{A(B-x)} = K \quad (6)$$

When A and B are equivalent equation (6) is replaced by equation (7)

$$\frac{dx}{dt} = K(A-x)(A-x) \text{ or } \frac{x}{t(A-x)A} = K. \quad (7)$$

Equations (6) and (7) have been found to hold very well experimentally, and hence we have excellent quantitative evidence that the hydrolysis of acetamide by aqueous solutions of acid takes place through the primary formation of the acetamide cation, $\text{CH}_3\text{CONH}_3^+$, and the subsequent hydrolysis of this salt.

But we can go a step further and show that the quantitative evidence obtained is not in harmony with the idea that an intermediate undissociated acetamide hydrochloride¹ is the chief substance that yields the end product. The complex acetamide cation and the chloride ions are in equilibrium with the undissociated salt, as expressed in equation (8)



or

$$(y-z)(C_{\text{Cl}}-z) = K_{\text{affn}} z,$$

or, since z is very small,

$$y \times C_{\text{Cl}} = K_{\text{affn}} C_{\text{salt und}} \quad (8)$$

in which, as above, y is the concentration of the acetamide cation, C_{Cl} that of the chloride ions, z , or $C_{\text{salt und}}$ that of the undissociated salt and K_{affn} the dissociation constant of the salt. By substituting the value of y , as obtained from (2) and (3), in equation (8) we get

$$(A-x)(B-x)B = K_{\text{affn}} \frac{K_b}{K_w} C_{\text{salt und}} \quad (9)$$

¹ Acree and Johnson: *Loc. cit.* Kastle: *Loc. cit.*

equation (9) which is accurate to within probably a few per cent. If now the undissociated salt were the chief substance being hydrolyzed and furnishing the end product we should have equation (10), giving the velocity of the reaction:



or

$$\frac{-dC_{\text{salt und}}}{dt} = K_{\text{trans}} C_{\text{salt und}} \times (C_{\text{H}_2\text{O}} - x). \quad (10)$$

But since, as above, $-dC_{\text{salt und}}$ is equivalent to dx , and for the value of $C_{\text{salt und}}$ we can always substitute the corresponding value in (9), we get equation (11)

$$\frac{dx}{dt} = \frac{K_{\text{trans}} K_b}{K_{\text{affn}} K_w} (A - x)(B - x)(C_{\text{H}_2\text{O}} - x)B. \quad (11)$$

When the solutions are dilute ($C_{\text{H}_2\text{O}} - x$) is practically constant and then we obtain equation (12) or

$$\frac{dx}{dt} = K(A - x)(B - x)B \quad \text{or} \quad \frac{1}{t(A - B)B} \log \frac{B(A - x)}{A(B - x)} = K. \quad (12)$$

When (A) and B are equivalent, equation (12) is replaced by equation (13),

$$\frac{dx}{dt} = K(A - x)(A - x)A \quad \text{or} \quad \frac{x}{t(A - x)A^2} = K. \quad (13)$$

When the proper quantitative data are substituted in equations (12) and (13) no constants are obtained. It is, therefore, clear that the undissociated acetamide hydrochloride is not the chief substance that yields the end products by hydrolysis. That the reasoning employed is correct was shown very satisfactorily in a previous paper¹ in which it was proved that hydrochloric acid causes the rearrangement of acetylchloraminobenzene into parachloracetanilide because the hydrochloric acid unites with the weak base, acetylchloramino-

¹ Acree and Johnson: *THIS JOURNAL*, **37**, 410; **38**, 258.

benzene and the undissociated salt rearranges. Since in the solution of the acetamide and the hydrochloric acid, the acetamide exists as free acetamide, acetamide cation and undissociated acetamide hydrochloride, one of these three forms of the acetamide must be the substance undergoing hydrolysis. Since the acetamide is hydrolyzed by water only extremely slowly and since the quantitative evidence of equations (12) and (13) is against the supposition that the undissociated salt is appreciably concerned in the reaction, it seems that the acetamide cation must be hydrolyzed by the water, just as the urazole anions react with neutral alkyl halides. Furthermore, the quantitative evidence is in entire harmony with the idea that the catalysis of acetamide by aqueous acids depends upon the hydrolysis of the intermediate acetamide cation formed by the union of some of the hydrogen ions with the weakly basic acetamide. This reaction may be complicated to a very slight extent by the hydrolysis of the free acetamide and the undissociated acetamide hydrochloride.

If the acetamide cation is hydrolyzed and equations (6) and (7) apply, it is evident that in dilute solutions the value of K should be the same for all concentrations. But it will be remembered that in equations (1) and (2) the values used for A and B in subsequent equations are really $(A-y)$ and $B-y$. When we divide by A in equation (7) we use a value larger than the more nearly correct value $(A-y)$ and K is slightly too small. As the values of A and B increase, the value of y increases and the factor $(A-y)$ becomes smaller and the value of K also smaller. We could predict, then, that if we were to use solutions of acetamide and hydrochloric acid in equivalent concentrations, 0.166 N, 0.333 N, 0.666 N, and 1.333 N, the values of K should be nearly constant but would show a slight decrease. If we refer to Tables I. to V., inclusive, we see that the values of K are 0.00027, 0.000223, 0.000205 and 0.00018. The application of equation (13) leads to the constants 0.0000144, 0.0000060, 0.00000275 and 0.0000012.

It is evident, then, that the use of equations (7) and (13) as approximate solutions is perfectly justifiable and leads to the conclusion that the acetamide cation, $\text{CH}_3\text{CONH}_2^+$, is the

chief substance undergoing direct hydrolysis, and not the free acetamide nor the acetamide hydrochloride, $\text{CH}_3\text{CONH}_2\text{Cl}$.

Furthermore, when the acetamide and hydrochloric acid are not in equivalent concentrations and equation (6) must be used, it is evident that a more nearly constant value for K should be obtained. From the reasoning in the preceding paragraph it is evident that the value of $(A-B)$ in equation (6) is exactly equivalent to the value we should have from equations (1) and (2), namely $(A-y)-(B-y)=(A-B)$. The errors in the logarithmic group are nearly canceled. We should expect to get a better value for K , then, from Tables VI., VII. and VIII., in which A and B are different. We actually get the values 0.000237, 0.000229, 0.000227 for K , which are not only in very good mutual agreement, but also agree closely with the average value of K in Tables I. to V., inclusive, 0.00022. It is, of course, understood that natural logarithms are used in finding the value of K , but the values AK and $(A-B)K$ are expressed in terms of Brigg's logarithms. The application of equation (12) to Tables VI., VII. and VIII. gives 0.000016, 0.0000062 and 0.0000030 as the values of K . It is evident, then, that the data obtained from Tables VI., VII. and VIII. agree very satisfactorily with the hypothesis that in the catalysis under consideration the acetamide cation is the chief substance undergoing hydrolysis and not the undissociated acetamide hydrochloride.

It will be seen from equations (4) and (5) that the value K is really $K_{\text{trans}} \frac{K_\delta}{K_w}$ and that the data in the present communication gives us neither the value of K_{trans} , the real velocity constant, nor that of K_δ . Since the present communication deals primarily with the question whether the acetamide cation or the acetamide hydrochloride yields the products of hydrolysis it is not necessary to know the value of K_{trans} and K_δ in order to get an approximate solution of the problem. The determination of the value of K_{trans} and K_δ will be the subject of the next communication on this subject.

An inspection of Tables I. to VIII., inclusive, shows that there seems to be a tendency for the value of the constant to rise

during the course of the reaction. This at once suggested, or rather confirmed, the idea that the reaction velocity might be accelerated by the ammonium chloride formed during the reaction. It will be recalled that many such catalytic accelerations, as well as retardations, of reactions by neutral salts have been recorded by Arrhenius,¹ Euler,² Koelichen³ and others. Experiments were, therefore, tried to ascertain whether the catalytic influence of other salts is positive or negative. The salts used were potassium chloride, sodium chloride, lithium chloride, aluminium chloride and chromium chloride. The lithium chloride seemed to act as a weakly negative catalyzer, as the value of K was lowered to 0.000215. Aluminium chloride seemed to be a weakly positive catalyzer, as the value of K was increased to 0.000236. The potassium chloride, sodium chloride and chromium chloride, however, seemed to act as energetic positive catalytic agents, since the value of K was raised to 0.00028, 0.00025 and 0.00027, respectively, by these salts. Finally, potassium chloride seemed to be a very energetic positive catalyzer for the catalysis of acetamide by acetic acid, as the value for K in the presence of potassium chloride was found to be 1.4×10^{-6} instead of 0.33×10^{-6} recorded by Ostwald for the value of K for acetic acid alone. These facts account to some extent probably for the slight rise in the constants during the hydrolysis of acetamide by the hydrochloric acid. The ammonium chloride formed in increasing amount may cause the velocity of hydrolysis of the acetamide by the hydrochloric acid and the acetic acid to be slightly greater than it would be if no ammonium chloride were present.

The value of K in Tables I. to XV. is given in terms of A (or B) = n (18.62) cc. nitrogen gas, liberated from 10 cc. of the solution by decomposition of the ammonium chloride formed in reaction. If A and B are expressed in terms of the concentrations used, that is, 0.166 N , 0.333 N , 0.666 N and 1.333 N , the value of K must be changed to correspond. K in Table VI., for example, changes from 0.000237 to 0.0264. In Table

¹ *Z. physik. Chem.*, **1**, 110; **4**, 226; **31**, 197.

² *Ibid.*, **33**, 348. *Ber. d. chem. Ges.*, **39**, 2726.

³ *Z. physik. Chem.*, **33**, 129.

XVIII. the values of K reckoned on both bases are given for all of the different dilutions.

After this article was handed to the editor an article by Crocker¹ on the hydrolysis of amides by hydrochloric acid came to our attention. Crocker's work verifies the above ideas concerning the catalysis of amides by acids in every detail.

EXPERIMENTAL.

The experiments were performed at a temperature of $65^{\circ} \pm 0.1$. The individual solutions of acetamide and of hydrochloric acid were made up at about 22° and then brought to 65° before the mixtures were made.

The analytical method used for following the reactions was that of Ostwald,² which is both accurate to within 2 or 3 per cent and very convenient. Measured volumes of the reaction mixture, from 2 cc. to 10 cc., were pipetted into a Lunge nitrometer containing a solution of 100 grams of sodium hydroxide and 25 cc. of bromine in 250 cc. water. A similar amount of water was added to the movable arm of the nitrometer when the gas reading was made. A correction was made for the absorption of the nitrogen by the hypobromite solution, the value of which was ascertained by measuring the nitrogen given off by the hypobromite and measured volumes of standard ammonium chloride solution.

In the experiments in which the catalytic action of other salts was studied the solid salt was added to the standard solution. A correction for the increase in volume by the salt and by the rise in temperature has been made in all the values of K . The volumes of gas are calculated at 0° and 760 mm. Correction was made for the vapor tension in the gas, in calculating A , B and x , and the necessary corrections were made in the value of K , which in all cases is given in natural logarithms.

¹ J. Chem. Soc., **84**, 593.

² *Loc. cit.*

Table I.

0.166 N HCl, 0.166 N acetamide, 65°. $A = 18.62$.

t	x	$A - x$	$\frac{x}{t(A - x)} = AK$
15			
30			
45	2.98	15.64	0.0042
60	4.13	14.49	0.0048
75	5.45	13.17	0.0055
90	6.14	12.48	0.0055
120	7.28	11.34	0.0055
150	7.93	10.69	0.0050
180	8.58	10.04	0.0048
240	8.72	9.90	0.0047

Average, 0.0050

 $K = 0.000268$

Table II.

0.333 N HCl, 0.333 N acetamide, 65°. $A = 37.23$.

t	x	$A - x$	$\frac{x}{t(A - x)} = AK$
15	4.15	33.08	0.0084
30	7.51	29.72	0.0084
45	9.81	27.42	0.0080
60	12.17	25.06	0.0081
75	14.19	23.04	0.0082
90	15.77	21.46	0.0082
120	18.65	18.58	0.0084
165	21.66	15.57	0.0085
240	24.50	12.73	0.0080

Average, 0.0083

 $K = 0.000223$

Table III.

0.666 N acetamide, 0.666 N HCl, 65°. A = 74.45.

<i>t.</i>	<i>x.</i>	A - <i>x.</i>	$\frac{x}{t(A-x)} = AK.$
15	13.31	61.14	0.0145
30	22.75	51.70	0.0147
45			
60	35.10	39.35	0.0148
75	40.14	34.31	0.0156
90	42.43	32.03	0.0144
120	47.72	26.73	0.0149
150	52.03	22.42	0.0155
180	55.01	19.44	0.0157
240	59.47	14.98	0.0165

Average, 0.0152

K = 0.000204

Table IV.

0.666 N acetamide, 0.666 N HCl, 65°. A = 74.45.

<i>t.</i>	<i>x.</i>	A - <i>x.</i>	$\frac{x}{t(A-x)} = AK.$
15	13.33	61.12	0.0145
30	23.22	51.23	0.0151
45	30.31	44.14	0.0153
60	35.14	39.31	0.0149
75	39.97	34.48	0.0155
90	43.52	30.93	0.0156
120	47.90	26.55	0.0150
150	52.22	22.23	0.0157
180	54.66	19.79	0.0153
240	59.73	14.72	0.0169

Average, 0.0154

K = 0.000206

Table V.

1.333 N HCl, 1.333 N acetamide, 65°. A = 148.90.

<i>t</i> .	<i>x</i> .	A - <i>x</i> .	$\frac{x}{t(A-x)} = AK.$
15	37.05	111.85	0.0221
30	61.56	87.34	0.0230
45	77.71	71.19	0.0243
60	87.24	61.66	0.0236
75	97.07	51.83	0.0250
90	105.10	43.80	0.0267
120	114.40	34.50	0.0276
150	122.80	26.10	0.0313
180	128.60	20.30	0.0352
240	131.10	17.80	0.0307

Average, 0.0269

K = 0.00018

Table VI.

0.333 N acetamide, 0.133 N HCl, 65°. A = 37.23, B = 14.89

<i>t</i> .	<i>x</i> .	A - <i>x</i> .	B - <i>x</i> .	$\frac{1}{t} \log \frac{B(A-x)}{A(B-x)} = \frac{(A-B)K}{(A-B)K}.$
15	1.71	35.52	13.18	0.0022
30	3.30	33.93	11.59	0.0023
45	4.42	32.81	10.47	0.0022
60	5.65	31.58	9.24	0.0023
75	6.31	30.92	8.58	0.0021
90	7.26	29.97	7.63	0.0022
120	8.86	28.37	6.03	0.0023
150	10.09	27.14	4.80	0.0024
180	11.21	26.02	3.68	0.0025
240	12.22	25.01	2.67	0.0024

Average, 0.0023

K = 0.000237

Table VII.

0.333 N HCl, 0.666 N acetamide, 65°. A = 74.45, B = 37.23.

<i>t.</i>	<i>x.</i>	A - <i>x.</i>	B - <i>x.</i>	$\frac{1}{t} \log \frac{B(A-x)}{A(B-x)} =$ (A - B)K.
15	7.22	67.24	30.01	0.0033
30	12.40	62.05	24.82	0.0032
45	17.76	56.69	19.46	0.0036
60	20.95	53.50	16.27	0.0036
75	24.39	50.06	12.83	0.0039
90	26.22	48.23	11.00	0.0038
120	29.88	44.57	7.34	0.0040
150	32.42	42.03	4.80	0.0043
180	34.00	40.45	3.22	0.0030
240	36.24	38.21	0.98	

Average, 0.00371

K = 0.000229

Table VIII.

0.666 N HCl, 0.333 N acetamide, 65°. A = 74.45, B = 37.23.

<i>t.</i>	<i>x.</i>	(A - <i>x.</i>)	(B - <i>x.</i>)	$\frac{1}{t} \log \frac{B(A-x)}{A(B-x)} =$ (A - B)K.
15	9.04	65.41	28.18	0.0043
30	13.51	60.94	23.71	0.0036
45	17.36	57.09	19.86	0.0035
60	20.34	54.11	16.88	0.0034
75	23.51	50.94	13.71	0.0036
90	25.58	48.87	11.64	0.0035
120	28.75	45.70	8.47	0.0036
150	32.00	42.75	5.22	0.0040
180	33.29	41.16	3.93	0.0040
240	34.36	40.09	2.86	0.0035

Average, 0.00368

K = 0.000227

Table IX.

0.333 N HCl, 0.333 N acetamide, 2 N KCl, 65°. $A = 37.23$.

t	x	$A - x$	$\frac{x}{t(A-x)} = AK$
15	4.70	32.53	0.0096
30	8.65	28.58	0.0101
45	11.94	25.29	0.0105
60	14.22	23.01	0.0103
75	16.34	21.16	0.0101
90	18.52	18.71	0.0110
120	21.36	15.87	0.0112
150	23.00	14.23	0.0108
180	24.95	12.28	0.0113
240	26.57	10.66	0.0104

Average, 0.0105

 $K = 0.00028$

Table X.

0.666 N HCl, 0.666 N acetamide, 4 N KCl, 65°. $A = 74.75$.

t	x	$A - x$	$\frac{x}{t(A-x)} = AK$
15	15.19	59.26	0.0171
30	26.85	47.60	0.0188
45	35.99	38.46	0.0208
60	40.71	33.74	0.0201
75	45.43	29.02	0.0209
90	46.80	25.85	0.0209
120	53.72	20.73	0.0216
150	57.24	17.21	0.0222
180	59.65	14.80	0.0224
240	62.84	11.61	0.0225

Average, 0.0207

 $K = 0.000278$

Table XI.

0.333 N HCl, 0.333 N acetamide, 2 N NaCl, 65°. $A = 37.23$.

t	x	$A - x$	$\frac{x}{t(A-x)} = AK$
15	4.35	32.88	0.0093
30	7.84	29.39	0.0089
45	10.70	26.53	0.0090
60	12.92	24.31	0.0086
75	14.17	23.06	0.0082
90	16.40	20.83	0.0087
120	16.61	20.62	0.0067
150	23.20	14.03	0.0110
180	23.04	14.19	0.0090
240	27.29	9.94	0.0114

Average, 0.0093

 $K = 0.00025$

Table XII.

0.666 N HCl, 0.666 N acetamide, 4 N NaCl, 65°. $A = 74.45$.

t	x	$A - x$	$\frac{x}{t(A-x)} = AK$
15	14.44	60.01	0.0158
30	25.71	48.74	0.0174
45	33.70	40.75	0.0184
60	38.87	35.58	0.0182
75	44.10	30.35	0.0194
90	46.68	27.77	0.0187
120	52.78	21.67	0.0203
150	55.94	18.51	0.0202
180	59.13	15.32	0.0214
240	63.33	11.12	0.0237

Average, 0.0193

 $K = 0.00026$

Table XIII.

0.333 N HCl, 0.333 N acetamide, 2 N LiCl, 65°. $A = 37.23$.

t	x	$A - x$	$\frac{x}{t(A - x)} = AK$
15	3.87	33.36	0.0077
30	7.04	30.19	0.0078
45	9.55	27.68	0.0076
60	11.37	25.86	0.0073
75	13.96	23.27	0.0080
90	15.25	21.98	0.0077
120	18.77	18.46	0.0084
150	20.97	16.26	0.0086
180	22.57	14.66	0.0086
240	24.52	12.71	0.0080

Average, 0.0080

 $K = 0.000215$

Table XIV.

0.333 N HCl, 0.333 N acetamide, 2 N AlCl₃, 65°. $A = 37.23$.

t	x	$A - x$	$\frac{x}{t(A - x)} = AK$
15	3.90	33.33	0.0078
30	7.18	30.05	0.0080
45	10.02	27.21	0.0082
60	12.25	24.98	0.0082
75	14.96	22.27	0.0090
90	16.33	20.90	0.0087
120	19.26	17.97	0.0089
150	21.80	15.43	0.0094
180	23.56	13.67	0.0096
240	25.70	11.53	0.0093

Average, 0.0088

 $K = 0.000236$

Table XV.

0.333 N HCl, 0.333 N acetamide, 2 N CrCl₃, 65°. A = 37.23.

<i>t.</i>	<i>x.</i>	A - <i>x.</i>	$\frac{x}{t(A-x)} = AK.$
15	6.08	31.15	0.0130
30	9.59	27.64	0.0115
45	11.80	25.43	0.0103
60	14.01	23.22	0.0101
75	15.41	21.82	0.0094
90	16.82	20.41	0.0092
120	20.31	16.92	0.0100
150	22.15	15.08	0.0098
180	23.49	13.74	0.0095
240	27.12	10.11	0.0112

Average, 0.0100

K = 0.000268

Table XVI.

0.666 N acetic acid, 0.666 N acetamide, 4 N KCl, 65°. A = 74.45.

<i>t.</i>	<i>x.</i>	A - <i>x.</i>	$\frac{x}{t(A-x)} = AK.$
120	0.92	73.53	104×10^{-6}
240	1.56	72.89	112×10^{-6}

Average, 108×10^{-6} K = 1.4×10^{-6}

0.666 N acetic acid, 0.666 N acetamide, 65° (Ostwald). A = 26.60.

<i>t.</i>	<i>x.</i>	A - <i>x.</i>	$\frac{x}{t(A-x)} = AK.$
14400	3.30	23.30	9.8×10^{-6}
28890	4.92	21.68	7.8×10^{-6}

Average, 8.8×10^{-6} K = 0.33×10^{-6}

Table XVII.
Conductivities, 18°.

Solution.	$\mu v.$	Difference.
0.1 N HCl	323.6	..
0.1 N HCl + 0.2 N acetamide	305.1	18.5
0.1 N HCl + 0.4 " "	287.0	18.1
0.1 N HCl + 0.6 " "	269.9	17.1
0.1 N HCl + 0.8 " "	259.1	10.8
0.1 N HCl + 1.0 " "	245.7	13.4
0.1 N HCl + 1.2 " "	235.5	10.2

Table XVIII.

Summary.

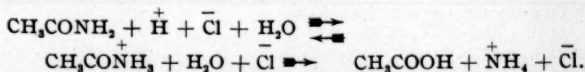
Table.	K.	K'¹
I.	0.000268	0.0300
II.	0.000223	0.0248
III.	0.000204	0.0228
IV.	0.000206	0.0231
V.	0.000180	0.0202
VI.	0.000237	0.0263
VII.	0.000229	0.0256
VIII.	0.000227	0.0254
IX.	0.000280	0.0315
X.	0.000278	0.0310
XI.	0.000250	0.0279
XII.	0.000260	0.0289
XIII.	0.000215	0.0240
XIV.	0.000236	0.0264
XV.	0.000268	0.0300
XVI.	0.0000014	0.000168

Conclusions.

The experimental work and the application of approximate equations in the present communication seem to confirm the hypothesis that in the hydrolysis of acetamide in aqueous hydrochloric acid the hydrogen ions first unite to some extent with the weak base acetamide and the acetamide cations

¹ K' is calculated on the basis of normal concentrations.

thus formed are hydrolyzed with the formation of acetic acid and ammonium ions:



This catalytic reaction seems to be analogous to the inversion of cane-sugar, hydrolysis of esters, formation of esters from urazoles and alkyl halides, and hydrolysis of oximes, discussed in a previous¹ communication.

JOHNS HOPKINS UNIVERSITY,
May 1, 1907.

REVIEWS.

ELECTROCHEMISTRY. I. Theoretical Electrochemistry and Its Physico-Chemical Foundations. By DR. HEINRICH DANNEEL, Privatdocent of Physical Chemistry and Electrochemistry in the Royal Technical High School of Aachen. Translated from the Sammlung Götschen by EDMUND S. MERRIAM, PH.D., Associate Professor of Chemistry in Marietta College, Ohio. New York: John Wiley & Sons; London: Chapman & Hall, Limited. 1907. pp. 181. Price, \$1.25 net.

Says the author, "Theoretical, or general chemistry, and electrochemistry are not separable; comprehension of the one presupposes knowledge of the other. Therefore, before we take up electrochemistry proper, we will go over some of the physical and physico-chemical principles which form the basis of our present ideas in the field of electrochemistry. We will then discuss the various theories of electrochemistry and give such illustrations as are necessary for their better comprehension. Experimental results and their applications as well as methods of measurement, etc., will be contained in the second volume. The third will be devoted to the technical applications."

As will be seen, this is but an introductory volume to the general subject. This will also appear from the Contents which comprises 7 chapters. Chapter I. deals with work, current and voltage; Chapter II. with chemical equilibrium statics, and kinetics; Chapter III. with the theory of electrolytic dissociation; Chapter IV. with conductivity; Chapter V. with electromotive force and the galvanic current; Chapter VI. with polarization and electrolysis; and Chapter VII. with the electron theory. An incomplete list of books dealing with electrochemistry is also given in this chapter.

¹ Acree and Johnson: *THIS JOURNAL*, 37, 410; 38, 258.

In discussing osmotic pressure it is unfortunate that the work of Morse and that of Berkeley should have been omitted. By discussing these recent investigations the book could have been brought more nearly up to date.

It is a little difficult to see just why Chapter II., on chemical statics and dynamics, should have been introduced at all into an elementary treatise on electrochemistry, and still less comprehensible why it should have found a place so near the beginning of the book. The best methods for determining transference numbers are omitted.

The best chapter in this first volume is the one that deals with the electromotive force of elements. The matters referred to above are, perhaps, after all, of minor importance, and the little book undoubtedly contains much valuable material presented in an attractive form.

The work of the translator seems to be satisfactory.

H. C. J.

THE VALUE OF PURE WATER. By GEORGE C. WHIPPLE. First edition. New York: John Wiley & Sons. 1907. pp. 84. Price, \$1.00.

In this interesting little volume the author estimates the money value of pure water to a community. The value of water to the consumer depends on its sanitary quality, its attractiveness and its hardness. The calculations of the relation between sanitary quality and money value to the community is based on the typhoid fever death-rate, and the value to the community of the average person dying from typhoid.

The book is well worth reading.

E. R.

PHYSIKALISCHE KRISTALLOGRAPHIE VOM STANDPUNKT DER STRUKTURTHEORIE. By ERNST SOMMERFELDT. Leipzig: Tauchnitz. 1907. pp. vii + 132.

This excellent little book is one of many works which come from the facile pen of its author. The initial idea seems to be to furnish a companion volume to the earlier work of the author on morphological crystallography and to the little introduction to chemical crystallography published by Professor Groth. The title of the work, however, is somewhat misleading, since the main part of the book is devoted to a rather full discussion of molecular crystallography or the theory of molecular structures. In the treatment the author follows in somewhat modified form Sohncke's theory and gives a very clear presentation of molecular structures according to this view. The treatment of the other physical properties of crystals which are grosser expressions of the molecular structures is relatively brief. The book might almost be called

"An Introduction to the Study of Crystal Structures." In spite of the title the book is an excellent treatment of the subject to which it is devoted and it is just the kind of a summary that chemists can use as a stepping stone to the more intricate papers by English crystallographers who are showing such possibilities in the study of the relations existing between the chemical constitution and crystallographic properties of many well-known substances.

The illustrations in the volume are exceptionally abundant, consisting mainly of photographs of numerous models which have been constructed to show the possibilities of molecular groupings according to the Sohncke theory. E. B. M.

MONOGRAPHIEN ÜBER ANGEWANDTE ELEKTROCHEMIE. VOL. XXV. DEUTSCHES PATENTRECHT FÜR CHEMIKER. VON DR. JULIUS EPHRAIM, Chemiker u. Patentanwalt, Berlin. Halle a. S.: Wilhelm Knapp. 1907. pp. 608. Price, M. 18.

The size of this volume would indicate that the subject has been thoroughly treated, how well, can only be judged by one who, like the author, is chemist and patent-lawyer.

E. R.

MONOGRAPHIEN ÜBER ANGEWANDTE ELEKTROCHEMIE. VOL. XXVI. ELEKTROMETALLURGIE DES EISENS. VON DR. BERNHARD NEUMANN, a. o. Professor a. d. Grossherzogl. Techn. Hochschule zu Darmstadt. Mit 89 Abbildungen. Halle a. S.: Knapp. 1907. pp. 176. Price, M. 7.

Professor Neumann is known as an authority in the field of elektrometallurgy, and this volume is interesting.

Electrolysis is not applied to the metallurgy of iron. The processes in use and those patented are all electrothermal, utilizing the heat of the arc to attain the temperatures required for one of the following reactions: A. The reduction of iron ores by carbon to metallic iron. B. The production of steel from pig iron in some modification of the Siemens-Martin furnace (1) by oxidizing the carbon of pig iron by iron ore; (2) by lowering the percentage of carbon by addition of scrap wrought iron; (3) by using ore and scrap. These are familiar processes, the only new thing being the use of the ore instead of generator gas. C. The production of iron compounds such as ferro-silicon, ferro-manganese, etc.

The author describes many processes for these applications of heat and gives statistical data for each process. The question is, do any of these processes pay or promise to pay in competition with those in common use?

For the processes under A, Neumann thinks that in the great iron-producing countries—the United States, England

and Germany—which have abundance of coal and iron ores, generally impure, the electric methods can never compete with the blast furnace. In countries, however, which have pure ores, cheap water power and little coal (Canada, South America, New Zealand), it is possible that the production of iron from the ore in the electric furnace may be a commercial success.

For the processes under B, Neumann concludes that some of the electrothermal processes not only can compete with the present method of making crucible steel but will eventually replace it.

As to the processes under C, these iron products are now made in Europe almost exclusively by the electrothermal process.

E. R.

WELLCOME'S PHOTOGRAPHIC EXPOSURE RECORD AND DIARY, 1907. New York and London: Burroughs, Wellcome & Co. 1907. Price, 50 cents.

This little pocket volume contains clear directions for using the publishers' photographic preparations, together with specimens of the pictures thus obtained. Useful numerical data, diary pages and an extremely ingenious exposure calculator are also included in the book, which cannot fail to be of service to those interested in the subject.

J. B. T.